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Impact of polyploidy on flavonoid constitution in *Narcissus tazetta* L

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Abstract. The data reported demonstrate the impact of polyploidy on plant phenotype and flavonoid constitution in *Narcissus tazetta*. Polyploidy induces increase in flower size and intensifies flower colour. The response of flavonoid constitution is variable. In some cases, flavonoid architecture does not register any change with the imposition of polyploidy. Whenever it does, the number of flavonoids is either increased or decreased. The probable explanation for such changes has been discussed.

Keywords. *Narcissus tazetta*; flavonoids; polyploidy

1. Introduction

Flavonoids are acknowledged indicators of phylogenetic kinship because they are so stable that ordinarily they are not influenced by changes in environmental conditions. The response of flavonoid constitution to numerical chromosome changes has been studied in very few cases (Iiyama and Grant 1972; Murray and Williams 1973; Crawford *et al* 1980). During the present study the phenotype and flavonoid constitution of a diploid and three triploid clones have been explored for comparison. The results form the text of present communication.

2. Materials and methods

Foliar and floral flavonoids of four varieties of *Narcissus tazetta* (listed in table 1) were screened through thin layer chromatography. The flavonoids were extracted separately from leaf lamina, and open flower in 1% HCl in methanol (2g per 1 ml). The separations were achieved on TLC plates coated with 0.6 mm thick layer of cellulose, using 5 μ l of test extract. The loaded plates were developed as proposed by Dass *et al* (1975). The flavonoid spots were detected under UV after spraying the plates with NH_3 and 1% NaOH and AlCl_3 solutions. At least three replicates were obtained for each taxon. The spots were grouped into three size categories namely, small (24–90 mm^2), medium sized (91–159 mm^2) and large (160–225 mm^2).

3. Results and discussion

Data on some quantitative characters of plant morphology of the four taxa are illustrated in the form of polygraph (figure 1), those on foliar and floral flavonoids are also included in tables 1 and 2 respectively. Of the four varieties, Canary Bird is a diploid with $2n = 20$, whereas the remaining three varieties are triploid with $2n = 30$.

Fifty one foliar flavonoids have been detected in the four taxa constituting the

Taxa	Spot numbers	Total no. of spots
Canary Bird	1s, 2s, 3s, 4s, 6s, 8m, 10s, 12s, 13s, 23s, 24s, 25s, 26s, 32s, 34s, 39s, 40s, 42s, 43s, 44s, 45s, 46m, 47s, 48s.	24
Kashmir Local	3s, 4s, 6s, 7s, 9m, 10s, 14s, 17s, 18s, 23s, 29s, 31s, 33s, 36s, 39s, 40s, 41s, 43s, 46m, 48s, 50s.	21
Kashmir Local Double	1s, 3s, 4s, 5s, 9s, 10s, 11s, 19m, 23m, 24s, 26s, 27s, 28m, 30s, 34s, 35s, 36m, 38s, 39s, 40s, 42s, 44s, 45s, 46s.	24
Soleil d'or	1m, 9m, 12m, 13s, 15s, 16m, 18s, 20s, 21m, 22s, 23m, 24s, 25s, 33m, 37m, 39s, 40s, 42s, 45m, 47s, 48s, 49s, 50s, 51s.	24

s, small; m, medium-sized,

experimental material. In individual taxa the number of spots ranges between 21 (Kashmir Local) and 24 (Canary Bird, Kashmir Local Double, and Soleil d'or). Three foliar flavonoid spots bearing nos. 23, 39 and 40 are common to all the four varieties (table 1).

Table 2 shows that the generalized chromatogram has 45 spots. The chromatograms of the four taxa were superimposed to obtain the generalized chromatogram. In individual varieties the number ranges from 15 (Kashmir Local) to 27 (Soleil d'or). Four flavonoids, represented by spot nos. 4, 10, 17 and 33, are common to the flowers of all the four varieties.

The influence of polyploidy on plant morphology was earlier studied in a variety of plants including narcissi (Stebbins 1971; Bose and Flory 1965; Smolskiy and Mankevich 1968; Raghuvanshi and Pathak 1975; Karihaloo 1977). In the taxa now studied, polyploidy has influenced vegetative as well as floral organs. Comparison of var Canary Bird (diploid) with Kashmir Local and Soleil d'or (both triploid) reveals that, while on the one hand, polyploidy has reduced plant height and scape length, on the other, it has boosted the flower size. Comparison between diploid Canary Bird and triploid Kashmir Local Double reveals a reverse trend. In this triploid, vegetative characters registered an increase in size but the floral features underwent diminution (figure 1).

Apart from the size changes, polyploidy also affects the colour of the flower. The colour deepens from light to deep yellow. In triploid vars Soleil d'or, Kashmir Local and Kashmir Local Double flowers are bicoloured. Development of novel colours has been observed in many other polyploids also (Smol'skiy and Mankevich 1968).

The impact of polyploidy on flavonoid pattern has been worked out in members of Poaceae and Rosaceae (Iiyama and Grant 1972; Kohli and Denford 1977). The polyploid varieties of *N. tazetta* have either only as many flavonoids as the diploid or fewer. The chromatograms of Canary Bird (2x) and Kashmir Local (3x) bear ten common spots. The triploid Soleil d'or shares twelve spots with the diploid and

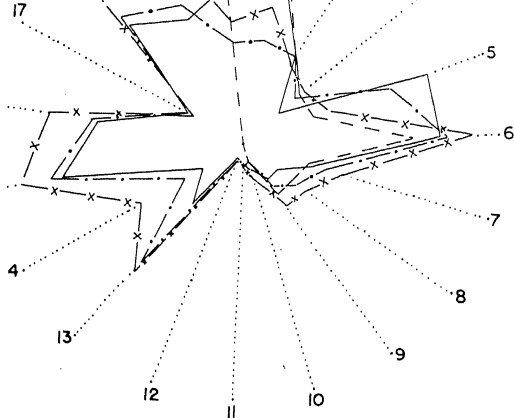


Figure 1. Polygraph illustrating variation in nineteen quantitative characters among four varieties of *N. tazetta* namely, Canary Bird (—), Kashmir Local (—x—), Kashmir Local Double (- - -), and Soleil d'or (—o—). 1. Plant height (1 cm = 7 cm), 2. Scape length (1 cm = 5 cm), 3. Number of leaves per plant (1 cm = 1 no.), 4. Breadth of leaf (1 cm = 0.5 cm), 5. Number of flowers, per inflorescence (1 cm = 1 no.), 6. Spread of flower, (1 cm = 0.5 cm), 7. Perianth lobe length (1 cm = 0.5 cm), 8. Perianth lobe breadth (1 cm = 0.5 cm), 9. Corona spread (1 cm = 0.5 cm), 10. Corona depth (1 cm = 0.5 cm), 11. Stamen length (1 cm = 0.5 cm), 12. Anther length (1 cm = 0.5 cm), 13. Pistil length (1 cm = 0.5 cm), 14. Style length (1 cm = 0.5 cm), 15. Polar axis of pollen (1 cm = 6 μ), 16. Equatorial axis of pollen (1 cm = 6 μ), 17. P/E ration (1 cm = 0.5), 18. Pollen size index (1 cm = 5), 19. Pollen volume (1 cm = 2400 μ^3).

Therefore appears closer to it. The flavonoids, which are common, exhibit quantitative differences as is reflected by the size of the spot they form on the chromatograms. For instance, spots 1, 12, 23 and 45 are small in chromatograms of Canary Bird but medium-sized in those of Soleil d'or. The increase in quantity can be attributed to the increased synthesis of these compounds in the polyploid taxa caused by the increase in number of genes coding for their synthesis.

Another effect of polyploidy is the synthesis of some novel flavonoids, the likes of which do not exist in the diploid. Appearance of novel flavonoids has been reported by Murray and Willams (1973) in polyploids of *Briza media*, and by Mangotra (1981) and

Canary Bird	1s, 2s, 4s, 6m, 9s, 10s, 12s, 14s, 15s, 16s, 17s, 20s, 21s, 22m, 28s, 30s, 32s, 33s, 34s, 35s, 39s.	21
Kashmir Local	2s, 4s, 10m, 15s, 17s, 23s, 24s, 27s, 28s, 31s, 33s, 34s, 38s, 44s, 45s.	15
Kashmir Local Double	1s, 3s, 4s, 5s, 7s, 8s, 10s, 13s, 17s, 20s, 21s, 22s, 25s, 29s, 30s, 32s, 33s, 36s, 40s, 42s, 43s.	21
Soleil d'or	1s, 2s, 4s, 6s, 10l, 11s, 14m, 15m, 16s, 17s, 18m, 19m, 20s, 22s, 23m, 24s, 26s, 28s, 31s, 33s, 34s, 36s, 37s, 38s, 39s, 41s, 42s.	27

s, small; m, medium-sized; l, large

Gupta (1983) in polyploids of *Crotalaria*. Their synthesis has been explained on the basis of the interactions among genes brought together in the polyploid taxa.

Like foliar, floral flavonoids also show three major trends; they increase, decrease or remains unaltered with the numerical increase in chromosome. The increase in number of flavonoids may be an outcome of recombination or presence of new gene combinations within the polyploids. The reduction in number of flavonoids can be explained on the grounds of simple dominance.

Comparison between the profiles of Canary Bird and Kashmir Local reveals that only eight floral flavonoid spots are common between the two. From among these, only one (represented by spot no. 10) shows increase in size. Similarly, Soleil d'or shares fifteen flavonoids with the diploid. Three of these (represented by spot nos. 10, 14 and 15) are produced in large quantity within the polyploids, but two others (represented by spot nos. 6 and 22) diminishes in quantity within the polyploids.

On the whole, flavonoid profiles of polyploid narcissi do not strictly match those of their diploid ally indicating their allopolyploid nature. The allopolyploid status is also borne out by the formation of some novel flavonoids. This conclusion is in line with that drawn on the basis of studies on their chromosome complement and the course of meiosis (Karihaloo 1977).

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References

- Bose S and Flory W S 1965 A cytological study of *Sprekelia formosissima*. Herbert; *The Nucleus* 8 115–128
 Crawford D J, Smith E B and Mueller A M 1980 Leaf flavonoid chemistry of *Coreopsis* (Compositae) section *Palmatae*; *Brittonia* 32 452–463

- Dass H C, Randhawa G S and Kour M 1975 Variation in flavonoid pattern in genus *Annona*; *Indian J. Hortic.* **32** 111–116
- Gupta M 1983 *Chemosystematics of some species of the genus Crotalaria* Ph.D. thesis, Jammu University.
- Iiyama K and Grant W F 1972 A correlation of nuclear DNA content and chromosome pattern in resolving genome relationships in *Avena*; *Can. J. Bot.* **50** 103–110
- Karihaloo J L 1977 Cytogenetic studies in some members of *Amaranthus* Ph.D. thesis, Jammu University
- Kohli B L and Denford K E 1977 A study of the flavonoids of the *Potentilla* species of America; *Can. J. Bot.* **55** 476–479
- Mangotra R 1981 *Cytomorphological and chemotaxonomical studies of some species of the genus Crotalaria* Ph.D. thesis, Jammu University.
- Murray B G and Williams C A 1973 Polyploidy and flavonoid synthesis in *Antirrhinum*; *Genetics* **243** 87–88
- Raghuvanshi S S and Pathak C S 1975 Polyploid breeding and possibility of *Antirrhinum drummondii* Hook; *Cytologia* **40** 355–363
- Smol'skiy N V and Mankevich O I 1968 Variation in flowers and inflorescences in decorative plants; *Vest. Akad. Navuk. BSSR, Ser. Biyal, Navuk* **4** 2–5
- Stebbins G L 1971 *Chromosome evolution in higher plants* (London: Academic Press)

Cotyledonary architecture in some Asteraceae

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Abstract. Cotyledonary blade is symmetrical in all the species of Asteraceae. Morphological features such as base, apex and margin vary from species to species. Venation pattern conforms to pinnate craspedodromous, pinnate camptodromous and acrodromous type. Higher order veins noticed are up to 5°. Primary vein is either stout massive, moderate or weak. The thickness of the veins gradually decreases from primary to higher order veins. Marginal ultimate venation is either incomplete, looped or fimbriate. Areoles are absent in *Eclipta prostrata*, *Chrysanthemum segetum* and *Calendula suffruticosa* sp *algarbiensis*. Vein endings are either simple or branched. In some species veins lack vein endings, which may or may not be associated with tracheids. Tracheids vary in their shape and arrangement in different species. Loop formation is observed. Isolated tracheids and extension cells are observed in *Calendula suffruticosa* sp *tomentosa*. All categories of veins are jacketed by parenchymatous bundle sheath.

Keywords. Anatomy; cotyledonary architecture; venation pattern; Asteraceae.

1. Introduction

The cotyledons are neglected organs in anatomical studies. Urban (1873) distinguished species of *Trigonella*, *Melilotus* and *Medicago* on the basis of cotyledonary features. Cotyledonary leaf architecture has been studied in the tribe Trifolieae of the Papilionaceae (Gupta 1978) and cultivars of cotton (Rao *et al* 1982). Murthy and Inamdar (1978) studied the effect of growth regulators on the cotyledonary architecture of *Lycopersicon esculentum*. Leaf architecture has been studied in some members of the Compositae (Banerjee and Deshpande 1973; Banerjee 1978a,b; Ravindranath and Inamdar 1982). The present work describes the cotyledonary architecture in 17 genera, 22 species and 3 sub-species, since no report exists.

2. Materials and methods

Seeds for the present study obtained from the Royal Botanical Gardens, Kew, Surrey, England, were grown in the botanical garden of this University at field conditions during winter. Mature cotyledons of the same age were collected at the seedling stage and cleared following the method of Rao *et al* (1980). Photomicrographs were taken with a Carl-Zeiss photomicroscope using Agfa film. Size of the cotyledons was measured using graph paper. The areole size, the number of veinlets entering in areole and the number of vein endings entering in areole were taken in five different fields of different cotyledons and the average value was recorded. Terminologies to describe cotyledonary architecture were adopted from Hickey (1973) and Hickey and Wolfe (1975).

Table 1. Qualitative features and numerical data on the cotyledonary venation patterns of some Asteraceae

Name of the taxa	Shape	Apex	Predominant tertiary vein origin angle	Marginal ultimate venation	Major venation pattern	Cotyledon area (mm) ²	No. of veins along one side of midrib	Range angle between 1° & 2° veins	No. of areoles/mm ²	No. of veinlets entering in tertiary areole/mm ²
<i>Calendula officinalis</i> L.	obl	ac	RR, RA	L	FB	38	6-7	40-45°	1	6
<i>C. maroccana</i> Ball	obo	ob	RR, AA	L	FB	24	6	45°	3	4
<i>C. stellata</i> Cav	obl	ob	AO, AA	L	FB	62	6-7	30-35°	2	4
<i>C. suffruticosa</i> Vahl	obo	ac	AA, AR	I	FB	44	7-8	40-45°	-	-
<i>sp. algarbiensis</i>										
<i>C. suffruticosa</i> Vahl	obo	ob	RR	I	FB	14	5	50-55°	1	6
<i>sp. lusitanica</i>										
<i>C. suffruticosa</i> Vahl	ell	ac	RR, RA	L	FB	16	8	45-55°	2	5
<i>sp. maritima</i>										
<i>C. suffruticosa</i> Vahl	ell	ac	RO, RA	I	FB	26	5-6	40-50°	1	3
<i>sp. tomentosa</i>										
<i>C. tomentosa</i> Desf	obl	ac	AA, RR	I	FB	40	8	80-85°	1	-
<i>Carthamus tinctorius</i> L.	obo	re	AO, RR	L	FB	92	3	20-30°	2	4
<i>Centaurea angustifolia</i> Mill	obo	re	AO, AR	I	FB	52	3-4	60-75°	1	4
<i>Chrysanthemum segetum</i> L.	obl	ac	AR	L	FB	4	5-6	40-45°	-	-
<i>Coreopsis lanceolata</i> L.	ell	ro	AA, RR	Fi	FB	20	6-7	35-40°	1	1

<i>ata</i>	ell	ro	RR, AR	Fj	FB	8	4-5	50-55°	-	-	-
	obl	re	RR, RO	I	acro	64	4	75°	2	3	6
<i>mus</i> L	ell	ac	RR, OR	I	sc	124	4-6	50-55°	2	6	8
<i>itus</i>	ell	ac	AA	I	sc	108	3-4	30-35°	1	4	6
<i>grey</i>											
<i>Nutt</i>	obl	ac	RR, RO	L	FD	54	5-6	40-45°	1	5	3
<i>umbens</i>	ell	ac	AR, RO	I	sc	96	5-7	40-50°	2	5	7
<i>mayya</i> &											
<i>aca</i>	obo	ob	AO, RR	I	FB	116	5	60°	1	5	6
<i>osus</i> L	ell	ob	AA, RR	I	FB	8	4-5	50-55°	2	5	1
<i>num</i> L	ell	ob	AA, RR	L	FB	306	7-8	55-60°	2	3	1
<i>L</i>	ell	re	RR, AA	I	FB	64	7-8	30-35°	1	6	8
	obl	ob	RR	I	FB	52	5	45-50°	2	3	3
<i>Blake</i>											
<i>dens</i> L	obl	ac	RR, AR	I	FB	12	3-4	30-35°	1	4	6
<i>rgens</i>	obl	ob	AO, RO	I	FB	64	4-6	40-45°	2	3	4
<i>ew</i>											

acrodromous; ell, elliptic; FB, fестоoned brochidodromous; Fj, fimbriate; I, incomplete; L, looped; ob, obtuse; obl, oblong; obo, obovate; obt, obtuse; re, retuse; edromous

3. Observations

3.1 Morphological description

Cotyledonary blade is symmetrical in all the species. Shape of the cotyledons is either oblong, obovate or elliptic. Apex is acute, obtuse, retuse or rounded. Base is mostly acute, but in *Eupatorium odoratum*, *Launaea procumbens*, *Tithonia rotundifolia* it is obtuse. Margin is entire in all the species except in *Helianthus annuus*, where it is serrate. The texture of the cotyledons is coriaceous in all the species studied. Species-wise qualitative features are charted in table 1.

3.2 Venation pattern

Venation pattern conforms to pinnate craspedodromous, pinnate camptodromous and acrodromous. In majority of the species cotyledonary blade belongs to pinnate type with a single primary vein serving as the source for other higher order veins. Secondaries after their origin from the primary terminate at the margin form the pinnate craspedodromous type. Before termination they divide and one of the branches joins with the superadjacent secondary and the other terminating at the margin is called semi-craspedodromous type (figure 1C). In *Coreopsis lanceolata* secondaries do not terminate at the margin and upturn acropetally to form loops known as brochidodromous type (figure 1D). These primary loops having a set of secondary loops outside the main brochidodromous type are called festooned brochidodromous type (figure 1A, B, E). In *Eupatorium odoratum* cotyledons have a single primary vein. Two strongly developed secondaries start from the base of the primary running towards the margin in a convergent fashion. Arches are not recurved at the base is said to be acrodromous type.

3.3 Venation

On the basis of the relative thickness of the veins and their origin they can be recognised into a number of size classes—primaries (1°), secondaries (2°), tertiaries (3°), quaternaries (4°) and quintenaries (5°). Primaries, secondaries and tertiaries form major venation pattern and quaternaries to other higher order veins form minor venation pattern.

3.4 Major venation pattern

The primary vein is the thickest vein originating from the base of the cotyledon and traverses towards its apex. Single primary vein is noticed in all the species. The nature of the primary vein is straight in most of the species. In *T. rotundifolia*, *C. suffruticosa* sp *algarbiensis*, *C. suffruticosa* sp *lusitanica*, *C. suffruticosa* sp *tomentosa* and *C. tomentosa* primary vein is sinuous (figure 1A, E). The size of the primary vein is either stout, massive, moderate or weak. The next order veins, secondaries originate from the primary either in alternate, opposite or sub-opposite fashion. The number of secondaries on one side of the midrib and the angle between primary and secondary vary from species to species and even within the same species. Secondary veins are

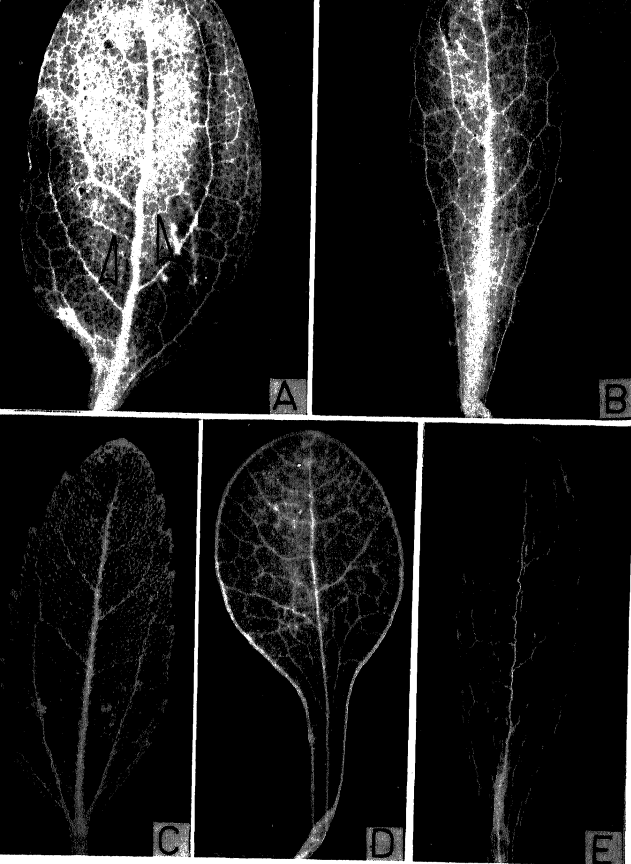


Figure 1. Cleared leaves showing morphological features and venation pattern in: (A) *T. rotundifolia* ($\times 9$). (B) *C. angustifolia* ($\times 6$). (C) *H. annuus* ($\times 7$). (D) *C. lanceolata* ($\times 9$). (E) *C. tomentosa* ($\times 6$).

tertiaries on both sides. The predominant tertiary vein origin angle on both exmedial and admedial side varies from acute-acute, acute-obtuse, obtuse-obtuse, acute-right angle, obtuse-right angle to right angle-right angle. The thickness of the veins gradually decreases from primary to tertiary. Intersecondary vein is observed in most of the species between two adjacent secondaries that originate from the primary. Intersecondary veins are simple (figure 1A, E at arrows).

3.5 Minor venation pattern

Tertiary veins give rise to quaternaries (4°) and quaternaries to quinternaries (5°). Higher order veins are observed upto 5° . The thickness of the veins gradually decreases from tertiaries to higher order veins. Minor veins are sinuous in *C. tomentosa* (figure 1E).

3.6 Marginal ultimate venation

Marginal venation is either incomplete, looped or fimbriate. The higher order veins after their branching and terminating freely within the margin is called incomplete type (figure 2A). In looped type higher order veins recurved to form loops inside the margin (figure 2B). In fimbriate type higher order veins after their ramification unite together to form a fimbrial vein which runs just inside the margin. Fimbrial vein is observed in *Chrysanthemum segetum* and *Coreopsis lanceolata* (figure 2C).

3.7 Areoles

The areoles are either imperfect or perfect. Areoles are absent in *Eclipta prostrata*, *C. segetum* and *C. suffruticosa* sp *algarbiensis*. The shape, size and number of veinlets entering into the areole vary from species to species and even within the same species. The shapes of the areoles may be quadrangular or irregular (figure 2D). Areoles are formed by all categories of veins.

3.8 Veinlets

The ultimate veins of minor venation which cross areoles are called veinlets. They may be simple or branched. Simple ones are either linear or curved (figure 2D) and branched ones, divide once or twice dichotomously (figure 2D). The number of veinlets, vein terminations and their absolute number per areole is not a constant feature, but vary from species to species and even within the same species. In *C. segetum*, *C. suffruticosa* sp *algarbiensis* and *E. prostrata* veins lack vein endings. Loop formation is observed in some species. Loops are formed within the areole and are formed by the union of ultimate vein endings (figure 2F) or a vein ending and a tracheid. In *C. suffruticosa* sp *tomentosa* loop-like structure is formed between the secondary vein (figure 2E). Loops are either triangular (figure 2F) or quadrangular (figure 2E).

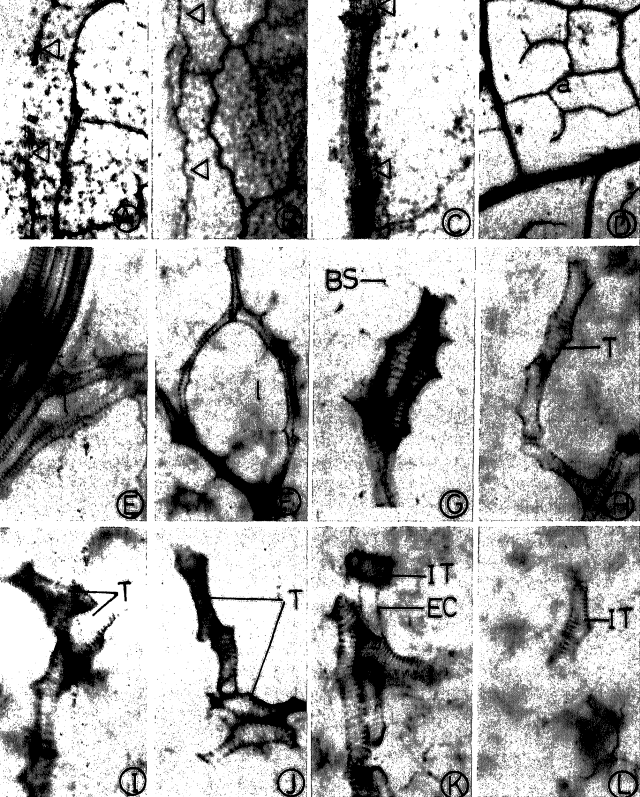


Figure 2. (A) Incomplete margin in *C. suffruticosa* sp. *tomentosa*. ($\times 120$). (B) Looped margin in *C. tomentosa* ($\times 40$). (C) Fimbriate margin in *C. lanceolata* ($\times 100$). (D) Areolation in *E. odoratum* ($\times 40$). (E, F) Loop formation in *C. suffruticosa* sp. *tomentosa*. E— $\times 320$, F— $\times 250$. (G) Presence of bundle sheath in *E. odoratum* ($\times 500$). (H) Uniseriate tracheids in *C. officinalis* ($\times 500$). (I). Uniseriate tracheids in *C. suffruticosa* sp. *tomentosa* ($\times 1120$). (J) Biseriate tracheids in *C. suffruticosa* sp. *tomentosa* ($\times 500$). (K, L) Isolated tracheids and extension cell in *C. suffruticosa* sp. *tomentosa*; K— $\times 480$, L—420.

Abbreviations: a, areole; l, loop; T, tracheids; IT, isolated tracheids; EC, extension cell, BS—bundle sheath.

Tracheids which lie freely in the mesophyll tissue of the areole are called isolated tracheids which may be short (figure 2K) or long (figure 2L). Sometimes tracheids connected with the veinlets by an extension cell are regarded as isolated as they are not connected with the vein by tracheary elements. Isolated tracheids are observed in *C. suffruticosa* sp *tomentosa*.

3.11 *Extension cell*

Extension cells are parenchymatous in nature found between the veins and tracheids which fail to differentiate as sieve and tracheary elements (figure 2K).

3.12 *Bundle sheath*

All categories of veins from major to minor are jacketed by bundle sheath. The bundle sheath cells are parenchymatous and are elongated in a direction parallel to the long axis of the veins (figure 2G).

4. **Discussion**

The size and shape of the cotyledons differ compared to the mature foliage leaves. The margin of cotyledons is mostly entire whereas that of the mature foliage leaves is serrate, dentate and entire. The venation pattern of cotyledons conforms to pinnate semi-craspedodromous, pinnate camptodromous with brochidodromous and festooned brochidodromous secondaries and acrodromous types. Similar types of venation pattern have been exhibited by the foliage leaves. However, cotyledons show decrease in the number of secondaries on either side of the primary vein as compared to the mature foliage leaves. The number of secondary veins on one side of the primary vein varies from species to species and even within the same species.

Gupta (1961) stated that the vein-islet and vein termination numbers are inversely proportional to the area of lamina. But Sehgal and Paliwal (1974), Singh *et al* (1976), Inamdar and Murthy (1978) and Rao *et al* (1982) concluded that there is no direct relationship between the size of the areole and the number of vein endings in different species as well as in the same species. The present observations also support the findings of these authors. But the number of free vein endings occurring in the areoles is less compared with those of mature foliage leaves because free vein endings are formed by the rupture of minor vascular network during development when the leaf expands as a result of mesophyll cell enlargement (Slade 1957, 1959) or there is a progressive differentiation of procambium from the ground meristem during the expansion of the lamina (Pray 1963).

The occurrence of isolated veins for the first time was reported by Kasapligil (1951),

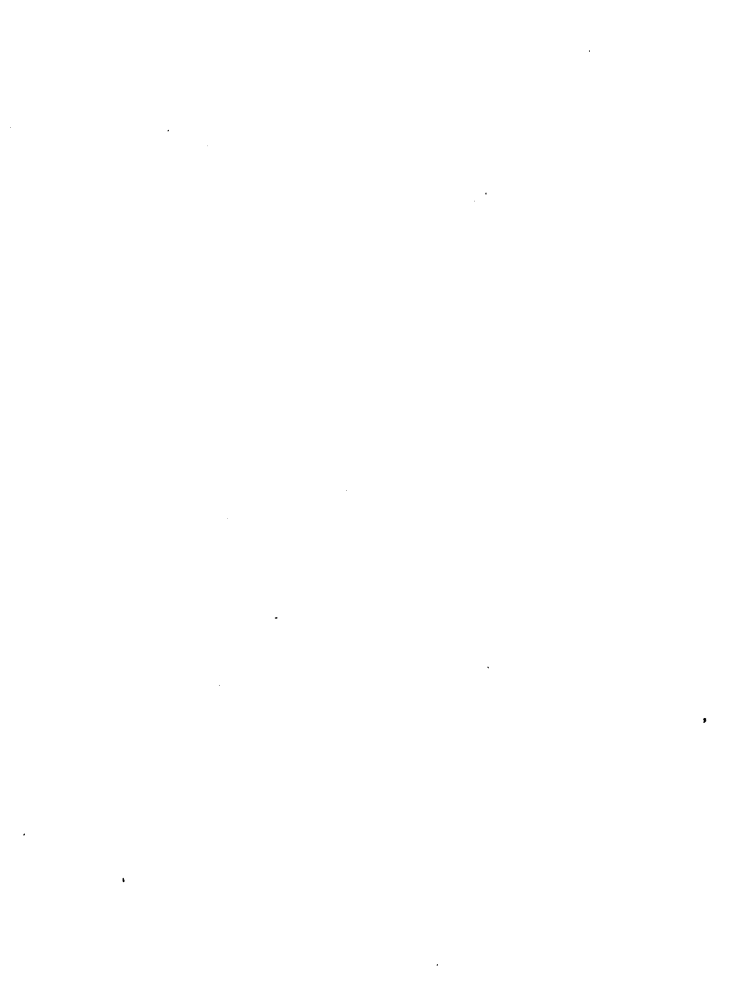
vein endings. (Pagnoulle 1962) reported the occurrence of extension cell between a vein and vein ending. Isolated tracheids and extension cell are observed in *C. suffruticosa* sp *tomentosa*. Veins which possess parenchymatous bundle sheath are referred to as ornamented by Sehgal and Paliwal (1974). Such ornamentations are observed in *E. odoratum*.

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We thank the Director, Royal Botanical Gardens, Kew, Surrey, England for kindly supplying the seeds. One of the authors (KR) thanks the UGC for a fellowship.

References

- Banerjee G 1978a Foliar venation of *Sonchus arvensis*; *Curr. Sci.* **47** 26–27
- Banerjee G 1978b Foliar venation and leaf histology of certain members of Compositae II. Members with combination Craspedodromy (C) *Amberboa ramosa* (Roxb.) Wagner (*Voluterella ramosa* Roxb); *J. Indian Bot. Soc.* **57** 338–342
- Banerjee G and Deshpande B D 1973 Foliar venation and leaf histology of certain members of Compositae; *Flora* **162** 529–532
- Gupta B 1961 Correlation of tissues in leaves. 1. Absolute vein-islet numbers and absolute veinlet termination numbers; *Ann. Bot.* **25** 65–70
- Gupta M 1978 Cotyledonary architecture in Trifolieae; *Acta Bot. Ind.* **6** 171–176
- Hara N 1962 On the types of foliage venation of *Dephne psuedomezereum* A. Gray; *Bot. Mag. Tokyo* **75** 107–113
- Herbst D 1972 Ontogeny of foliar venation in *Euphorbia forbesii*; *Am. J. Bot.* **59** 843–850
- Hickey L J 1973 Classification of the architecture of dicotyledonous leaves; *Am. J. Bot.* **60** 17–33
- Hickey L J and Wolfe J A 1975 The basis of angiosperm phylogeny-vegetative morphology; *Ann. Misso. Bot. Gard.* **62** 538–589
- Inamdar J A and Murthy G S R 1978 Leaf architecture in some Solanaceae; *Flora* **167** 265–272
- Kasapliligil B 1951 Morphological and ontogenetic studies of *Umbellulara californica* Nutt and *Lourus nobilis* L.; *Univ. Calif. Pub. Bot.* **25** 115–240
- Murthy G S R and Inamdar J A 1978 Effect of colchicine and morphactin on the cotyledonary venation of *Lycopersicon esculentum* Mill var *angulata*; *Phyton* **19** 337–347
- Pray T R 1963 Origin of vein endings in angiosperm leaves; *Phytomorphology* **13** 60–81
- Ravindranath K and Inamdar J A 1982 Leaf architectural studies in Asteraceae I; *Pak. J. Bot.* **14** 143–154
- Rao V S, Shenoy K N and Inamdar J A 1980 Clearing and staining technique for leaf architectural studies; *Microscopica Acta* **83** 307–311
- Rao Venigella S, Inamdar J A and Murthy G S R 1982 Cotyledonary architecture in cultivars of cotton; *J. Econ. Tax. Bot.* **3** 335–341
- Sehgal L and Paliwal G S 1974 Studies on the leaf anatomy of Euphorbia II Venation patterns; *Bot. J. Linn. Soc.* **68** 173–208
- Singh V, Jain D K and Sharma M 1976 Leaf architecture in *Salix*; *J. Indian Bot. Soc.* **55** 140–150
- Slade B F 1957 Leaf development in relation to venation as shown in *Cercis siliquastrum* L., *Prunus serrulata* Lindl and *Acer pseudoplatanus* L.; *New Phytol.* **56** 281–300
- Slade B F 1959 The mode of origin of the vein endings in the leaf *Liriodendron tulipifera* L.; *New Phytol.* **58** 299–305
- Urban I 1873 Prodrum einer Monographie der Gattung *Medicago* L. *Verh. Bot. Ver. Prov. Erandenb* **15** 1–185



Seed morphology in five species of *Biophytum* DC (Oxalidaceae)

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Abstract. Seeds of *Biophytum* viz *B. helenae*, *B. intermedium*, *B. petersianum*, *B. reinwardtii* and *B. sensitivum* are brownish, small (0.87 to 1.63 mm long), tuberculate and show distinct orientations of ridges and furrows and crystalliferous epidermal cells. On the basis of these characters a key is proposed to delimit the five species of *Biophytum*.

Keywords. *Biophytum*; seed morphology; taxonomic significance.

1. Introduction

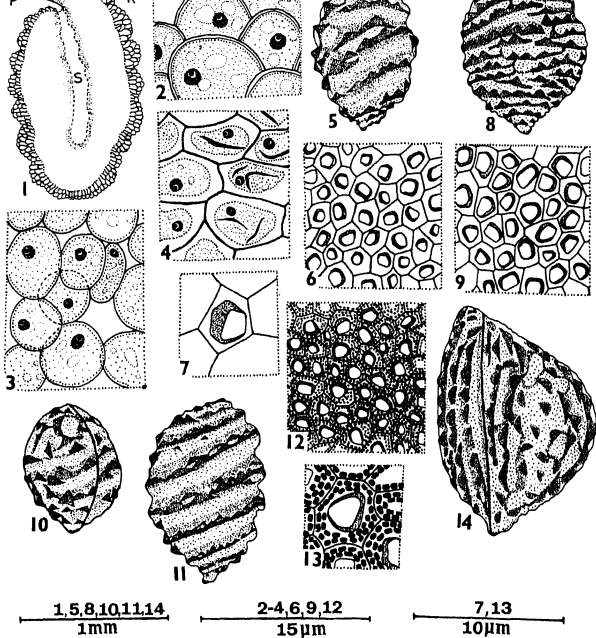
Biophytum DC (Oxalidaceae) comprises about 70 species distributed in the tropics (Willis 1973). Although its seed characters have been used for, taxonomic purposes (Gamble 1957; Veldkamp 1971; Corner 1976), the seed microcharacters have not been studied. We describe the testa characters of five species of *Biophytum*.

2. Materials and methods

Seeds of *Biophytum helenae* Busc & Musc, *B. intermedium* Wight, *B. petersianum* Klotzch, *B. reinwardtii* Edgw & Hook and *B. sensitivum* L. were locally collected and also obtained from Jardin Botanique, Strasbourg, France. The structure of the pellicle was studied from material collected from the freshly dehiscent capsule, and this paper gives the details of the testa from a portion of seed coat separated from the water-soaked seeds and mounted in 1 % glycerine. Exomorphic features were studied using a Meopta binocular microscope. Seed length and breadth were measured using ocular and stage micrometers and an average of 25 seeds of each species was considered.

3. Observations and discussion

In the species studied the mature seeds are brownish, 0.87–1.63 mm long and 0.75–1.16 mm broad. They are triangular, ovoid to spherical with the posterior and either blunt or pointed. As in *Averrhoa carambola* (Oxalidaceae), in *Biophytum* the mature seeds are individually enclosed in a pellicle. The pellicle is colourless, fleshy and papillate and presents a wavy outline (figure 1). The outer surface of the pellicle contains papillae which are oval to spherical cells containing a distinct nucleus and rich cytoplasm. The inner surface of the pellicle comprises polygonal cells which contain one or two lenticular to sickle-shaped structures (figures 2–4). At maturity the seed frees itself from the pellicle through an anterior longitudinal slit developed just below the placental projection (figure 1).



Figures 1-14. 1-4. *Biophytum reinwardtii*, P = placenta, R = ridge, S = longitudinal slit. 1. Entire pellicle after the ejection of seed. 2. A ridge portion of the pellicle. 3, 4. Cells from outer and inner surfaces of the pellicle respectively. Note the characteristic lenticular structures in cells of the inner surface. 5-7. *B. helenae* Entire seed, testa part and single cell with prismatic crystal respectively. 8, 9. *B. intermedium* and structure of ridge. 10. *B. petersianum*. 11-13. *B. reinwardtii*, testa part and single cell with prismatic crystal respectively. 14. *B. sensitivum*.

tuberculate seeds showing scattered tubercles (*B. petersianum* and *B. sensitivum*) and (2) non-tuberculate seeds showing specifically oriented ridges and furrows (*B. helenae*, *B. intermedium* and *B. reinwardtii*). Figures 5-14 are representative illustrations of the two types of seeds. The following key is proposed for the identification of the five species.

Seed tuberculate

Seed triangular, 1.5-1.7 mm long, 1.0-1.2 mm broad, testa dimorphic, epidermal cells crystalliferous, tubercle cells not crystalliferous *B. sensitivum*

monomorphic both epidermal and tubercle cells crystalliferous	<i>B. petersianum</i>
Seed non-tuberculate	
Seed spherical, ridges and furrows transverse, testa cells crystalliferous	<i>B. intermedium</i>
Seed ovoid, ridges and furrows spiral, testa cells contain granules and crystals	<i>B. reinwardtii</i>
Seed sub-ovoid, ridges and furrows oblique, testa cells crystalliferous	<i>B. helenae</i>

The seeds of *Biophytum* are described as arillate (Lawrence 1964; Veldkamp 1971; Willis 1973) but Corner (1976) regards the seeds of Oxalidaceae (except *Dapania* orth) as non-arillate. Our study supports Corner's observation. Both optical and SEM the closely related genus *Oxalis* have been found to be of taxonomic value (Bahadur al 1983). This study shows that seed and seed-coat characters in *Biophytum* serve as significant taxonomic traits and merit further investigations.

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References

Bahadur B, Vijaya Bhasker K and Farooqui S M 1983 LM and SEM studies of seed-coat in five species of *Oxalis* L (Oxalidaceae); *Proc. Indian Natn. Sci. Acad.* **B49** 348–353

Corner E J H 1976 *The seeds of Dicotyledons*; (Cambridge: Univ. Press)

Hamble J S 1957 *Flora of the Presidency of Madras* (Calcutta: Botanical Survey of India) Vol. 1

Lawrence G H M 1964 *Taxonomy of vascular plants*; (New Delhi: Oxford IBH Pub. Co.)

Veldkamp J F 1971 Oxalidaceae In: *Flora Malesiana*; (ed) C J Ser (Netherlands: N. V. P. Noordhoff-Groningen) vol. 7 pp.151–178

Willis J C 1973 *A dictionary of the flowering plants and ferns*; (Cambridge: Univ. Press) 8th ed.

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Abstract. Various types of stomatal abnormalities like persistent stomatal initials, single guard cells, degenerated guard cells and contiguous stomata with contiguity in different directions were recorded due to the treatment of different growth substances. Growth substances also altered the stomatal size, epidermal cell size, stomatal frequency, index and length-to-breadth ratio. It seems that the effect of these substances starts from the stomatal ontogeny and continue up to the last phase of development leading to such variations.

Keywords. Stomatal character; *Gossypium hirsutum*; growth substances

Introduction

Stomatal shape and arrangement on the leaf lamina are influenced by growth substances (Subrahmanyam *et al* 1972; Kasat 1979). These changes in stomata may be useful for a plant to grow in an unsuitable environment as stomata are the main outlets for water loss. Therefore, it is necessary to screen the growth substances for the purpose. In the present study, efforts have been made to find out the effects of 2,4,5-T, GA_3 , ascorbic acid and 2,3,5-T on stomatal characters in *Gossypium hirsutum* var H-777.

Materials and methods

Experiments to find out the effects of various concentrations of 2,4,5-trichlorophenoxy acetic acid, ascorbic acid (AA), gibberellic acid (GA_3) and 2,3,5-triiodobenzoic acid (TIBA) on stomatal characters in *Gossypium hirsutum* var H-777, the seeds of which were procured from Hissar Agricultural University, Hissar, were performed in the Botanical Garden of the University. The plants were raised in unglazed earthenware pots of twelve inch diameter filled with a mixture of garden soil and farmyard manure (2:1 ratio) and were kept under natural conditions of light and temperature. Each selected concentration of a growth substance was sprayed on 10 plants. Thus the experimental design consisted of seventeen sets each with 10 pots. Aqueous solutions having 50, 100, 200 and 500 mg/l of AA, GA_3 and TIBA and 10, 25, 50 and 100 mg/l of 2,4,5-T were prepared. In all these solutions and distilled water, 2 ml of soapnut extract were added as wetting agent. The plants were sprayed when they were 1 month old having six leaves. It was repeated later 3 times at 10-day intervals. Mature leaves which emerged after the foliar spray treatments, were plucked, washed and fixed in FAA. Peels

stomata and epidermal cells per mm^2 were calculated. Length and breadth of stomata and epidermal cells were also measured. Stomatal index was calculated as defined by Salisbury (1927, 1932) viz $100S/(E + S)$, where E is the number of epidermal cells and S is the number of stomata over a given surface per unit area. Each reading is an average of 10 plants in a single treatment. To find out the relative effect of growth substances on length and breadth of the stomata, their ratio was calculated separately for both the surfaces.

3. Observations

Plants which were sprayed with 100 ppm 2,4,5-T and 500 ppm TIBA died. In the rest of the treatments, plants continued to grow.

(i) *Effect on epidermis*: The shape of the epidermal cells varies. It is either rectangular, polygonal, isodiametric or elongated. Cell walls are mostly straight (figure 1J) and sinuous (figure 1A).

(ii) *Effect on stomata*: Leaves are amphistomatic; stomata are randomly oriented throughout the epidermis. Majority of stomata are anomocytic type (figure 1A). In this type the epidermal cells surrounding the stoma are four in number and equal in size. They are so oriented that two epidermal cells lie parallel and two perpendicular to stoma. Some diacytic (figure 1B) and anisocytic (figure 1C) stomata were also observed. Following variations were observed in stomata in the control and treated leaves.

Persistent stomatal initials were encountered in TIBA 200 ppm (figure 1F). They showed slightly thicker wall with dense cytoplasm. Stomata with one guard cell only, were observed in GA_3 200 ppm and 500 ppm and 50 ppm 2,4,5-T (figure 1D). In GA_3 500 ppm, in some stomata, guard cells were found degenerated leaving only a thickening around the pore (figure 1I). In GA_3 200 and 500 ppm treatments, unequal guard cells, one being bigger and the other smaller, were also observed (figure 1H). Various oriented contiguous stomata like juxtaposed (GA_3 200 and 500 ppm, figure 1K), superimposed (TIBA, 200 ppm; figure 1G) and at right angles to each other (TIBA 200 ppm, figure 1E). Stomata with abnormal big pore were recorded in 200 ppm GA_3 (figure 1L).

(iii) *Effect on epidermal cell size* (table 1): The epidermal cells present on abaxial and adaxial sides had a dimensions of $49.95 \times 35.80 \mu$ and $48.82 \times 37.33 \mu$ respectively. In 50 ppm 2,4,5-T, the epidermal cell size decreased while in ascorbic acid and TIBA, the size increased. GA_3 increased the epidermal cells in length.

(iv) *Effect on stomatal size* (table 1): In a normal leaf, the stomata of the adaxial side are bigger than those of abaxial side. 2,4,5-T, GA_3 and ascorbic acid treatments, increased the stomatal size on both the leaf surfaces. In TIBA treatment, stomata remained smaller in comparison to those of control.

(v) *Effect on frequency of epidermal cells* (table 2): The number of epidermal cells in a mm^2 of a normal leaf is 135.59 on abaxial side and 123.35 on the adaxial side. This frequency was increased by 2,4,5-T treatment only. The rest of the growth substances

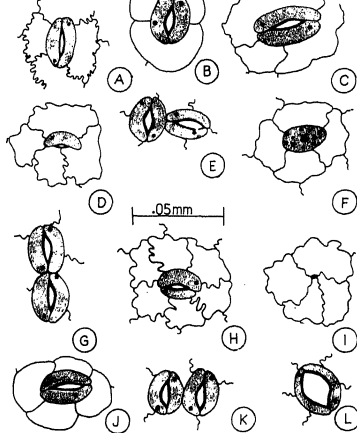


Figure 1. A-L. Effect of growth substances on the stomata of *Gossypium hirsutum* var H-77

used i.e. ascorbic acid, GA_3 and TIBA decreased the frequency of epidermal cells on both the leaf surfaces. The maximum decrease was in GA_3 .

(vi) *Effect on frequency of stomata* (table 2): In control leaf, the stomatal frequency was 40-49 on the abaxial surface and 30-13 on the adaxial surface. Except TIBA all the growth substances used in the study increased the stomatal frequency.

(vii) *Effect on stomatal index* (table 2): This index comes out 22-99 and 19-63 on the abaxial and adaxial sides of control leaf. However, its value for both abaxial and adaxial surfaces was more than these in all the treatments except TIBA.

(viii) *Effect on stomatal length/breadth ratio* (table 1): This ratio shows that stomata were broadly elliptic on both surfaces of a normal leaf. They were broadly elliptic on the abaxial side in 10, 25 and 50 ppm 2,4,5-T but were narrow elliptic on the adaxial side. In 50 ppm and 500 ppm AA, stomata were broadly elliptic but were narrow elliptic in 100 and 200 ppm ascorbic acid on the abaxial side. On the adaxial side, they were narrow elliptical in 50, 100 and 200 ppm ascorbic acid and broadly elliptic in 500 ppm. In GA_3 , stomata appeared narrow elliptic on the abaxial side of 200 and 500 ppm treatments and on the adaxial side of 200 ppm. In the rest of the concentrations of GA_3 , the stomata were broadly elliptic on both the leaf surfaces. Broadly elliptic shape of the stomata remains unchanged on both surfaces of the leaf in TIBA except on the abaxial side in 100 ppm where the stomata become narrow elliptic.

4. Discussion

Growth substances have affected the shape and size of stomata and epidermal cells variously. In the present investigation, abnormalities like persistent stomatal initial,

Table 1. Effect of different growth substances on epidermal characters in *Gossypium hirsutum* var H-777

Treatment	Concen- tration (ppm)	Epidermal cell size (μ)						Stomatal size (μ)						Length/breadth Ratio*	
		Abaxial			Adaxial			Abaxial			Adaxial				
		Length	Breadth	Length	Breadth	Length	Breadth	Length	Breadth	Length	Breadth	Length	Breadth	Abaxial	Adaxial
Control	0	49.95 ± 3.24	35.80 ± 2.04	48.82 ± 3.23	37.73 ± 0.89	29.97 ± 0.84	19.98 ± 4.31	31.63 ± 6.32	21.64 ± 4.23	1.49	1.46				
2,4,5-T	10	47.45 ± 1.27	33.30 ± 1.08	43.29 ± 4.02	37.63 ± 0.75	29.97 ± 1.87	20.81 ± 2.81	35.96 ± 5.43	23.31 ± 3.21	1.44	1.54				
	25	39.96 ± 2.04	34.10 ± 1.07	43.29 ± 5.01	29.64 ± 0.76	33.00 ± 2.83	21.98 ± 3.91	38.96 ± 4.23	24.14 ± 2.63	1.58	1.61				
	50	39.13 ± 1.09	30.80 ± 3.24	36.63 ± 4.02	30.64 ± 0.83	32.47 ± 1.87	22.48 ± 2.06	36.30 ± 3.24	22.48 ± 2.23	1.44	1.62				
	100	-	-	-	-	-	-	-	-	-	-	-	-	-	
AA	50	49.95 ± 5.72	36.63 ± 0.94	46.62 ± 3.01	39.96 ± 0.75	29.97 ± 2.34	21.65 ± 2.08	35.96 ± 3.23	19.98 ± 3.21	1.38	1.80				
	100	53.28 ± 6.03	35.79 ± 0.24	49.12 ± 4.02	43.29 ± 0.76	33.30 ± 4.32	21.65 ± 3.07	36.63 ± 4.24	22.48 ± 2.23	1.54	1.63				
	200	52.45 ± 4.02	42.45 ± 3.24	49.95 ± 5.07	42.29 ± 0.83	39.13 ± 7.52	20.81 ± 0.94	42.29 ± 1.23	26.64 ± 3.32	1.88	1.59				
	500	53.28 ± 3.04	49.45 ± 4.27	53.28 ± 6.02	43.29 ± 0.75	34.13 ± 2.24	23.31 ± 0.74	36.29 ± 2.21	24.97 ± 2.19	1.46	1.45				

GA ₃	50	59.94 ±2.09	26.64 ±2.63	53.28 ±2.03	29.97 ±0.82	29.97 ±3.32	19.98 ±1.24	28.30 ±2.32	23.31 ±2.18	1.50	1.21
	100	79.92 ±0.92	29.97 ±1.62	52.54 ±4.09	28.97 ±2.34	32.63 ±3.12	22.48 ±1.23	33.30 ±3.32	25.80 ±2.62	1.45	1.29
	200	73.26 ±0.56	23.31 ±0.94	53.28 ±5.02	29.97 ±2.94	36.63 ±4.06	21.64 ±1.22	36.63 ±3.43	23.31 ±3.12	1.69	1.57
	500	69.93 ±0.43	36.63 ±0.93	56.61 ±3.02	36.63 ±1.63	36.09 ±5.02	22.98 ±2.22	35.23 ±4.42	26.64 ±3.14	1.57	1.32
TIBA	50	53.28 ±0.42	33.30 ±0.94	48.47 ±4.02	38.63 ±0.92	29.13 ±4.05	19.98 ±1.06	29.97 ±2.39	19.98 ±1.08	1.46	1.50
	100	49.62 ±0.82	39.30 ±0.93	53.29 ±5.03	38.80 ±3.73	25.97 ±3.06	19.18 ±1.05	26.64 ±2.64	19.98 ±1.02	1.36	1.33
	200	53.29 ±0.93	38.30 ±0.72	50.79 ±4.32	36.46 ±4.62	24.97 ±2.05	14.15 ±2.07	23.31 ±3.62	19.98 ±2.07	1.76	1.17
	500	—	—	—	—	—	—	—	—	—	—

* If equal to or more than 1.5 = broad elliptical. If less than 1.5 = narrow elliptical.

Treatment	Concentration (ppm)	Frequency of epidermal cells (mm ⁻²)		Frequency of stomata (mm ⁻²)		Stomatal index	
		Abaxial	Adaxial	Abaxial	Adaxial	Abaxial	Adaxial
Control	0	135.59 ±15.29	123.35 ±20.20	40.49 ±4.32	30.13 ±2.08	22.99	19.63
2,4,5-T	10	131.83 ±20.87	129.94 ±21.22	49.34 ±3.56	35.78 ±1.07	27.23	21.59
	25	154.43 ±10.56	137.48 ±13.33	54.24 ±7.32	42.75 ±1.05	25.99	23.72
	50	148.77 ±9.82	131.83 ±14.32	56.49 ±8.26	45.19 ±1.05	27.52	25.53
	100	-	-	-	-	-	-
	50	133.71 ±20.75	120.53 ±20.22	46.70 ±3.25	33.89 ±2.04	25.89	21.95
AA	100	123.73 ±10.42	123.73 ±16.72	48.96 ±4.32	37.66 ±0.92	28.35	23.07
	200	124.29 ±10.42	125.61 ±14.12	53.11 ±3.23	40.87 ±2.02	29.94	27.23
	500	118.64 ±5.82	99.81 ±8.07	54.61 ±3.25	39.36 ±1.98	31.52	28.28
	50	130.32 ±20.75	101.69 ±20.92	50.85 ±4.75	38.98 ±1.78	28.06	27.71
	100	97.36 ±6.56	92.28 ±7.58	48.59 ±6.78	45.20 ±1.72	33.16	32.88
GA ₃	200	99.81 ±8.98	79.09 ±6.02	48.96 ±8.89	41.43 ±1.73	32.91	34.58
	500	90.39 ±7.08	74.76 ±8.09	50.85 ±9.35	47.46 ±0.96	35.66	36.83
	50	133.71 ±15.74	120.03 ±20.02	36.16 ±4.30	35.22 ±2.32	21.29	20.68
	100	120.53 ±13.24	112.99 ±18.75	25.80 ±2.20	20.53 ±4.01	17.63	15.37
	200	105.27 ±12.64	118.64 ±20.02	19.40 ±1.05	22.03 ±3.32	15.56	15.66
TIBA	500	-	-	-	-	-	-

single guard cell which according to Inamdar *et al* (1974) develop either directly from meristemoid or by degeneration of one of the guard cells, degenerated guard cells and contiguous stomata in which contiguity was in different directions, were observed on both the surfaces of the leaf in *Gossypium hirsutum* var H-777 due to treatment with various doses of growth substances. These findings support the earlier findings of Inamdar *et al* (1974) who also observed abnormalities in stomatal shape due to treatment of growth substances. Various explanations like cytoplasmic heterogeneity (Morgan 1934), extrinsic factors (Bunning 1952), intrinsic instability (Dehnel 1961), genetic factor (Kasat 1979; Sharma and Dunn 1968) have been given for the formation of abnormal stomata.

As a general effect, 2,4,5-T, GA₃ and ascorbic acid increased the stomatal size, index

higher concentrations, reduced stomatal size, frequency and index on both the surfaces. Earlier Gangadhara and Inamdar (1975) observed increased stomatal frequency in GA and IAA treated *Cucumis sativus* seedlings.

Owing to smaller size of epidermal cells in 2,4,5-T treatment, the frequency of these cells increased. However, ascorbic acid and TIBA increased the size of epidermal cells and thus their frequency declined. Similar was the effect of GA₃ and these findings agree with Murty *et al* (1976) who also observed enlargement of epidermal cells due to GA treatment.

Ratio of stomatal length to breadth which was calculated to find out the effect of different growth substances on stomatal shape showed that in *G. hirsutum*, the stomata are broadly elliptic on both surfaces of a control leaf and remain the same on the abaxial surface in 10 and 50 ppm 2,4,5-T, 50 and 500 ppm ascorbic acid, 50 and 100 ppm of GA₃ and TIBA, but they became narrow elliptic in 25 ppm 2,4,5-T, 100 and 200 ppm ascorbic acid, 200 and 500 ppm GA₃ and 200 ppm TIBA. On adaxial surface they were broadly elliptical in control, 500 ppm AA, all concentrations of GA₃ and TIBA but became narrow elliptic in the rest of the treatments.

It is clear from the present discussion that growth substances produce several variations in the morphology of stomata as well as in their frequency, index etc, which are possible only if the ontogeny was affected. Therefore, the findings support Gangadhara *et al* (1977) who was of the opinion that differentiation of a meristemoid into stomatal complex may be controlled by the specific intrinsic factors at the region of meristemoid and these factors are disturbed by the exogenous application of growth substances which ultimately may lead to such aberrant developments. The view of Kasat (1979) who considered that growth substances in general do not have any effect on ontogeny of stomata but they affect their morphology in the last phase of their development did not get any support from the present findings except that the morphology would have been altered at the later phase of their development leading to variations in stomatal shape.

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References

- Bunning E 1952 Morphogenesis in plants, In *Survey of biological progress*. (ed.) G S Avery (New York: Academic Press) vol. 2, pp. 105-140
- Dehnel G S 1961 Abnormal stomatal development in the foliage leaves of *Begonia aridicaulis*; *Am. J. Bot.* **48** 129-133
- Gangadhara M and Inamdar J A 1975 Action of growth regulators on the cotyledonary stomata of *Cucumis sativus* L structure and ontogeny; *Biol. Plantarum*. **17** 292-303
- Gangadhara M, Rao T B, Inamdar J A and Patel R M 1977 Effect of growth regulators on the structure and development of cotyledonary and hypocotyledonary stomata of *Gossypium hirsutum* L var Digvijay; *Phyton*. **18** 9-28
- Inamdar J A, Gangadhara M and Sanjeeva Rao M 1974 Effect of growth regulators on stomatal structure and ontogeny in the cotyledons of *Cucurbita maxima* Duch; *Geobios*. **1** 113-117
- Kasat M L 1979 Morphogenetic effect of various growth regulators on the foliar stomata of *Vigna sinensis*; *Geophytologia* **9** 102-107

- Morgan T H 1934 *Embryology and Genetics* (New York: Springer-Verlag)
- Murty Y S, Prakash G and Poonia O P 1976 Effect of gibberellic acid on cotyledonary epidermis of *Crotalaria juncea* L; *Sci. Cul.* **42** 438–440
- Salisbury E J 1927 On the causes and ecological significance of stomatal frequency with special reference to woodland flora; *Philos. Trans. R. Soc. London* **B216** 1–65
- Salisbury E J 1932 The interrelations of soil, climate and organisms and the use of stomatal frequency as an integrating index of the water relation of the plant; *Beih. Bot. Zbl.* **99** 402–420
- Sharma G K and Dunn D B 1968 Effect of environment on the cellular features in *Kalanchoe fedchenkoii*; *Bull. Torrey. Bot. Club.* **95** 464–473
- Subrahmanyam D, Parabha M H, Mehta P M and Shah G L 1972 Morphogenetic effects of different growth regulators on the cotyledonary stomata of *Glycine max.* In *Biology of L and Plants*, (eds) V Puri *et al* (Meerut: Sarita Prakashan) pp. 160–172

A proposed system for virulence designation in India.

2. *Puccinia graminis* f sp *tritici*

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Abstract. A new virulence analysis system for the wheat stem rust pathogen (*Puccinia graminis* f sp *tritici*) based on three sets of differentials is suggested. Set A consists of eight single gene lines for stem rust resistance—*Sr8*, *Sr9b*, *Sr9e*, *Sr11*, *Sr13*, *Sr28*, *Sr30* and *Sr37* (*SrTl-2*) and set B has five standard differentials—Marquis, Einkorn, Kota, Reliance and Khapli, and one supplementary differential—Charter. Set O includes two susceptibles, Agra Local (Wheat) and a local barley, one universal resistant and six cultivars. It shows the behaviour of selected cultivars to stem rust flora while variations in the pathogen are identified on sets A and B. The new nomenclature is based on binary notation and decanary values.

Keywords. Virulence analysis; stem rust; *Sr* gene; *Puccinia graminis tritici*.

1. Introduction

Pathogen populations are in a dynamic state and within a few years of large scale cultivation, new resistant cultivars may become susceptible. After the wheat stem rust epidemic which swept the United States in 1953, caused by a new strain of race 15, it became known that the pathogen (*Puccinia graminis* f sp *tritici*) contained an indefinite number of biotypes (Stakman *et al* 1956). These differed in pathogenicity and other physiological characters. Besides their identification on 12 standard differentials (Stakman and Levine 1922), additional cultivars were capable of further separating them. This led to the conclusion that it would be difficult to devise an objective system for classifying *P. graminis tritici* races until adequate genetic knowledge was established (Stakman *et al* 1962).

In accordance with the gene-for-gene hypothesis (Flor 1942) virulence analysis systems have been revised in many countries (Watson and Luig 1963, Johnson *et al* 1972; Roelfs and McVey 1974). Basic information about race specific interaction and wheat genotypes to Indian stem rust races was collected by Patra *et al* (1976). Based on studies on the interaction of stem rust races and wheat genotypes, a new system for virulence analysis is proposed on the pattern of a leaf rust system suggested earlier (Nagarajan *et al* 1983).

2. Materials and methods

2.1 Constitution of proposed sets

The new system consists of three host sets A, B and O. Sets A and B each allow for nine entries, but at present only eight are proposed for set A and six for set B. The lines with known *Sr* genes for stem rust resistance are arranged in descending order from those

wheat with *Sr13* is susceptible to the most; others are intermediate (table 1). The seeds of near isogenic/substitution lines for stem rust resistance were collected from Dr R N Sawhney, IARI, New Delhi, who initially obtained from Dr R A McIntosh, University of Sydney, Australia. The details of wheat lines are given in table 1. A few lines in Marquis and other backgrounds were obtained from Dr A P Roelfs, University of Minnesota, USA. Further details may be collected from original source if necessary.

A compilation of fifty years of virulence analyses has shown that of 12 standard differentials of the Stakman set, only six are useful in India. Some have the same genes for resistance *e.g.* Arnautka, Mindum and Spelmar (*Sr9d*) and Kubanka and Acme (*Sr9g*). Therefore, only five standard and one supplementary, differentials with known genes or gene combinations have been retained in set B. They include Marquis (*Sr7b*, *Sr18*, *Sr19*, *Sr20*), Einkorn (*Sr21*), Kota (*Sr7b*, *Sr18*, *Sr28*), Reliance (*Sr5*, *Sr16*, *Sr18*, *Sr20*), Khapli (*Sr7a*, *Sr13*, *Sr14*) and Charter (*Sr11* + ?). This set along with lines possessing *Sr9e* and *Sr11* included in set A, identifies all the races according to the old system and also forms a link between the old and the new systems of nomenclature.

The O set has nine entries including a susceptible wheat check (Agra local), a line resistant to all known races, a barley cultivar, five selected cultivars of *T. aestivum* and one cultivar of *T. durum* presently grown in various agroclimatic zones of the country. The set O has no role in race identification but surveys the behaviour of various important cultivars to the pathogen population.

Table 1. Details of entries included in set A, B and O for virulence identification of the stem rust pathogen (*Puccinia graminis* f sp *tritici*)

Set-A	Near isogenic line/variety	Set-B	+ CI number
<i>Sr13</i>	Khapstein/10 Marquis	Marquis (<i>Sr7</i> , <i>Sr18</i> , <i>Sr19</i> , <i>Sr20</i>)	CI 3641
<i>Sr9b</i>	CS/KF 2B	Einkorn (<i>Sr21</i>)	CI 2433
<i>Sr11</i>	1 <i>Sr11Ra</i> (W 3015)	Kota (<i>Sr7b</i> , <i>Sr18</i> , <i>Sr28</i>)	CI 5878
<i>Sr28</i>	Kota; = <i>Sr28</i> (CH 71)	Reliance (<i>Sr5</i> , <i>Sr16</i> , <i>Sr18</i> , <i>Sr20</i>)	CI 7370 ^b
<i>Sr8</i>	1 <i>Sr8Ra</i> (W 3384)	Charter (<i>Sr11</i> + ?)	E 557
<i>Sr9e</i>	Vernstein	Khapli (<i>Sr7a</i> , <i>Sr13</i> , <i>Sr14</i>)	CI 4013
<i>Sr30</i>	Festiguay		
<i>Sr37*</i>	Line W		
Set-O	Pedigree of cultivars		
Agra Local	Local wheat selection		
WL 711	(S308 × Chris) × Kalyansona		
Sonalika	(11-54-388-An) × (Yt 54 × NI 1008)		
	LR		
Lok-1	Not known		
<i>Sr24</i>	<i>Sr24</i> 3D/Ag (Isogenic line)		
Bijga yellow	Local × Gaza		
NI-5439	RFPM80 × NP 7103		
Nilagiri	H 41-3 × (HD 1962-E 4870 × K-65)		
Barley	Local selection		

+ Cereal Investigation accession number, US Dept. of Agriculture.

* *SrTt-2* renamed as *Sr37*.

ounded by a necrotic border, 3 = uredinia medium to large without necrosis and
= when uredinia large, coalescent. In the present system there was no instance where
e mixed (X-type) reaction occurred. Infection types 0, 0₁, 1, and 2 denote resistance
(R), and 3 and 4 susceptibility (S).

Results and discussion

After repeated tests of all currently maintained races on sets A and B, the reactions
aving high levels of consistency, are shown in table 2. The reactions of races 42B and
22 were identical and were not differentiated on set A. On set B, these races are
istinguished on Reliance. Since, the *Sr28* line and Kota (*Sr7b*, *Sr18*, *Sr28*) gave
fferent reactions to race 21A-1, Kota has also been retained. Identical reaction of
ota and wheat line *Sr28* to all virulences except 21A-1, shows that the resistance of
Sr28 is probably due to the presence of another gene in it, not common with Kota.
The new system has a number of merits over the old system. About 10 single gene
es for stem rust resistance are used revealing their effectiveness to various races. The
gher the race designation number, the more widely virulent the strain is. The new
stem works well at temperatures of 15–25°C. However the reaction should be
autiously recorded during cloudy weather as races 122 and 295 produce susceptibility
n wheats with *Sr28*, races 24 and 24A on wheats with *Sr30* and race 40A on those with
-37. At the higher temperature of 30°C races 15 and 42 show avirulence for *Sr8*.

1 How to name the virulence?

A number of virulence analysis systems based on lines with known genes for stem rust
istance have been proposed by various workers (Watson and Luig 1963; Roelfs and
cVey 1974). In these systems, naming of a race may become lengthy and at times
fficult to decode. Since the binary notation system proposed by Habgood (1970) and
e European system for new nomenclature of the yellow rust pathogen have merits of
recision (Johnson *et al* 1972), the current proposal for designating Indian races of
graminis tritici has been tailored on the same pattern.

Set O has no role in designation system. In sets A and B, lines are arranged serially.
ach entry is given a decanary value of 2⁰ to 2⁸ (table 3). The number in each set is
imited to nine entries so that the maximum decoded decanary value does not exceed
000.

According to the binary notation system, when a pathogen isolate is tested on the
arious lines, a resistant response is denoted as 0 and a susceptible response as 1. Hence
actions of race 14 on set A would be denoted by the binary notation 00001000,
dicating that the *Sr8* line is susceptible whereas all others are resistant. The sequential
nary values are multiplied with their respective decanary values and are totalled. The
lue for set B is determined on a similar basis. Therefore, race 14 gets values of 16 on
t A and 2 on set B. Accordingly, race 14 is redesignated 16G2, where the letter G

Table 2. Reactions on near isogenic lines and standard differentials with known genes when tested with stem rust races and their new nomenclature.

Sr genes	11A	14	15	17	21	21A-1	21A-2	24	24A	34	40	40A	42	42B	117	117	117	122	184	295
															A	A-1				
Sr13	S	R	R	S	S	R	S	R	S	R	R	R	S	S	R	R	S	S	S	S
Sr9b	S	R	S	R	R	R	S	S	R	S	R	S	S	S	R	R	S	S	R	S
Sr11	R	R	R	R	S	R	R	R	S	R	S	S	S	S	S	S	S	S	S	S
Sr28	S	R	S	S	S	R	S	R	R	S	S	S	R	R	R	R	R	R	R	R
Sr8	R	S	S	R	R	S	R	S	R	S	S	S	S	R	R	R	R	S	R	R
Sr9e	R	R	S	R	R	R	R	R	R	R	S	S	R	R	S	S	S	R	S	R
Sr30	S	R	R	S	R	R	S	R	R	R	S	R	R	R	R	R	R	R	R	R
Sr37	S	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
<i>Standard differentials</i>																				
Marquis	S	R	S	S	S	S	S	S	S	S	S	S	S	S	S	R	R	S	S	S
Einkorn	S	S	S	R	R	R	R	S	S	R	R	R	S	S	S	S	S	S	R	S
Kota	S	R	S	S	S	S	S	R	R	S	S	S	R	R	R	R	R	R	R	R
Reliance	S	R	S	R	R	R	R	S	S	S	S	S	R	R	R	R	R	S	R	S
Charter+	R	R	R	R	R	S	R	R	R	S	R	S	R	R	R	R	S	R	R	R
Khapli	R	R	R	R	R	R	R	R	R	R	R	R	S	S	R	R	R	R	R	S

New nomenclature: 11A = 203G15, 14 = 16G2, 15 = 58G15, 17 = 73G7, 21 = 9G5, 21A-1 = 20G21, 21A-2 = 75G5, 24 = 18G3, 24A = 5G18, 34 = 26G13, 40 = 104G13, 40A = 62G29, 42 = 19G35, 42B = 7G35, 117 = 37G3, 117A = 36G2, 117A-1 = 38G18, 122 = 7G11, 184 = 53G1, 295 = 7G43

+ Supplementary differential.

Table 3. Procedure for determination of race designation. Examples for old races 14 and 40A.

Details	Set-A/ <i>Sr</i> lines								Set-B					
	13	9b	11	28	8	9e	30	37	MAR	EIN	KOT	REL	CHA	KHP
Decanary value	2 ⁰	2 ¹	2 ²	2 ³	2 ⁴	2 ⁵	2 ⁶	2 ⁷	2 ⁰	2 ¹	2 ²	2 ³	2 ⁴	2 ⁵
Decoded decanary value	1	2	4	8	16	32	64	128	1	2	4	8	16	32
Reaction of race 14	R	R	R	R	S	R	R	R	R	S	R	R	R	R
Binary value	0	0	0	0	1	0	0	0	0	1	0	0	0	0
Value for each line	0	0	0	0	16	0	0	0	0	2	0	0	0	0
Total value for race 14 = 16G2														
Reaction for race 40A	R	S	S	S	S	S	R	R	S	R	S	S	S	R
Binary value	0	1	1	1	1	1	0	0	1	0	1	1	1	0
Value for each line	0	2	4	8	16	32	0	0	1	0	4	8	16	0
Total value for race 40A = 62G29														

MAR = Marquis, EIN = Einkorn, KOT = Kota, REL = Reliance, CHA = Charter, KHP = Khapli.

separates the two values and indicates that the pathogen is *P. graminis tritici*. Similarly, the value for race 40A becomes 62G29.

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References

- Flor H H 1942 Inheritance of pathogenicity of *Melampsora lini*; *Phytopathology* **32** 653-669
- Johnson R, Stubbs R W, Fuchs E and Chamberlain N H 1972 Nomenclature of physiologic races of *Puccinia striiformis* infecting wheat; *Trans. Br. Mycol. Soc.* **58** 475-480
- Habgood R M 1970 Designation of physiologic races of plant pathogen; *Nature (London)* **227** 1268-1269
- Nagarajan S, Nayar S K and Bahadur P 1983 The proposed brown rust of wheat (*Puccinia recondita* f sp *tritici*) virulence analysis system; *Curr. Sci.* **52** 413-416
- Patra N K, Sawhney R N and Chopra V L 1976 Effectiveness of known *Sr* genes for seedling resistance against Indian stem rust races in wheat; *Curr. Sci.* **45** 70-72
- Roelfs A P and McVey D V 1974 Races of *Puccinia graminis* f sp *tritici* in USA during 1973; *Pl. Dis. Repr.* **58** 608-611
- Stakman E C and Levine M N 1922 The determination of biologic forms of *Puccinia graminis* on *Triticum* sp; *Minn. Agri. Exp. Tech. Bull.* **8** 1-10
- Stakman E C, Loegering W Q and Stewart D M 1956 Identification of physiologic races of *Puccinia graminis tritici*; Supplement No. 1, *Cooperative Rust Laboratory, St. Paul, Minnesota* pp. 1-11
- Stakman E C, Stewart D M and Loegering W Q 1962 Identification of physiologic races of *Puccinia graminis*

Response of groundnut (*Arachis hypogaea* L) to combined inoculation with *Glomus mosseae* and *Rhizobium* sp

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Abstract. The adequate level of inoculum of a vesicular-arbuscular mycorrhizal fungus, *Glomus mosseae*, required for copious mycorrhizal formation in groundnut (*Arachis hypogaea* L) in sterilized soil was found to be one gram dried root powder from infected plants per kg soil and this level was used subsequently in an inoculation study. In two cultivars of groundnut, combined inoculation with a *Rhizobium* sp and *G. mosseae* resulted in synergistic effect on the formation of mycorrhiza and nodulation when compared to separate inoculations. The combined inoculum resulted in fairly good response of the plants in terms of dry weight, uptake of nitrogen and phosphorus.

Keywords. *Glomus mosseae*; groundnut (*Arachis hypogaea* L); *Rhizobium* sp; vesicular-arbuscular mycorrhiza.

1. Introduction

Inoculation of different crop plants with VA mycorrhizal fungi and *Rhizobium* was found to have synergistic beneficial effect on nodulation, nitrogen fixation and plant growth (Crush 1974; Daft and El-Giahmi 1976; Mosse *et al* 1976; Bagyaraj *et al* 1979). This increase was attributed to better phosphorus nutrition of legumes as a result of mycorrhizal formation. Smith and Daft (1977) showed that time is an important factor in the development of the tripartite symbiosis between lucerne, *Rhizobium meliloti* and *G. mosseae*. Asimi *et al* (1980) observed considerable decrease in infection by *G. mosseae* in soybeans at higher levels of phosphate (0.5 or 1 g $\text{KH}_2\text{PO}_4/\text{kg}$). Groundnut was shown to form mycorrhiza with *Glomus mosseae* (Nic. & Gerd.) Gerd. & Trappe in this locality (Rao and Parvathi 1982). The present paper reports the effect of inoculants of *Glomus mosseae* and *Rhizobium* sp on mycorrhizal formation and nodulation in two cultivars of groundnut. Initially, the inoculum potential of the VAM fungus for its maximum development in groundnut was also ascertained.

2. Materials and methods

The soil used in all experiments was a phosphorus deficient (P, 0.0045 %) latosol with a pH of 8.1. The potting medium consisted of a 2:1 soil:sand mixture taken in 20-cm earthen pots and autoclaved. In order to determine the inoculum potential of *Glomus mosseae*, different levels of inoculum (0.05, 0.1, 0.2, 0.4, 0.6, 0.8, 1.0, 2.0, 4.0 and 6.0 g kg^{-1} potting medium) in the form of dried and powdered groundnut roots colonized by *G. mosseae* were thoroughly mixed. Unsterilized and uninoculated potting mixture served as control. For each inoculum level three pots were maintained and seeds of groundnut cv TMV-2 were sown. The pots were maintained in an open

shade at a temperature range of 27 to 31°C. After 60 and 100 days of growth, five plants were removed for each inoculum level. The roots were cleared with KOH and stained with trypan blue (Phillips and Hayman 1970) and percentage root length infection as well as the number of vesicles or spores cm^{-1} root were determined, in a random sample of 25 to 50 one cm pieces, by the root-slide technique (Nicolson 1959). At the last sampling, plant dry weight and pod number were estimated for each inoculum level. Nitrogen and phosphorus in plant tissues were determined by the methods of Jackson (1967) and Fogg and Wilkinson (1958), respectively. The data on per cent root infection and the number of vesicles/spores were statistically analysed as detailed earlier (Parvathi *et al* 1985).

In another experiment, a soil-sand mixture was taken in 20-cm earthen pots and sterilized. Seeds of groundnut (cvs TMV-2 and Robut 33-1) were inoculated with peat culture of *Rhizobium* sp (1 g of peat/100 seeds) obtained through the courtesy of Dr P T C Nambiar, ICRISAT, Hyderabad, and sown in pots containing sterilized soil-sand mixture. The number of viable cells was 10^9 g^{-1} peat culture. The inoculum of *G. mosseae* in the form of dried infected groundnut roots (0.1 % w/v) was added to the potting medium, ten days after the addition of *Rhizobium* sp, which provided the following combinations: (a) uninoculated control, (b) *G. mosseae* alone, (c) *Rhizobium* sp alone and (d) *Rhizobium* sp + *G. mosseae*. For each combination, three pots with 10 seeds sown in each pot were maintained. After 60 and 90 days of inoculation, five plants were taken out from the three pots and the number and weight of nodules per plant, the per cent root infection and the number of vesicles or spores cm^{-1} root length were determined. The plant dry weight, nitrogen and phosphorus content were estimated in triplicate samples and the values are represented as averages.

3. Results

A high level of mycorrhizal development was observed in the plants raised in unsterilized uninoculated potting medium (table 1). The plants raised in sterilized potting medium with inoculum levels of zero to 0.1 g (kg^{-1} soil) failed to develop mycorrhiza. There was a gradual increase in mycorrhizal infection at inoculum levels of 0.2 g to 1.0 g. Beyond this no further significant increase occurred (figure 1). A comparison of results (table 1 and figure 1) shows that there was very little change in per cent infection between 60 and 100 days. By 60 days, infection in the root systems reached a plateau, at higher levels of inoculum. However, sporulation by the fungus increased significantly at 4.0 and 6.0 g levels of inoculum. The plant dry weight, estimated 100 days after inoculation, was greater at the inoculum level of 1 g than at lower levels (0 to 0.8 g). The growth response was almost the same at higher levels as at 1.0 g level. The number of pods formed was more (5 pods plant^{-1}) with 1.0 g inoculum than at the lower levels (0 to 0.8 g) which varied on the average from 1.8 to 3.6 per plant. There was no further increase in pod number at the higher inoculum levels (2, 4 and 6 g) when compared to 1.0 g level. The per cent of nitrogen and phosphorus was higher in the plants raised with 1.0 to 6.0 g inoculum than at lower levels of inoculum.

Inoculum level (g powdered roots kg ⁻¹ soil)	VA mycorrhiza, after 60 days of plant growth		Plant analysis, after 100 days of growth		
	Root length infected (%)	Vesicles or spores cm ⁻¹ root	N (%)	P (%)	Pod number
0	0	0	2.72	0.5	1.8
0.05	0	0	2.96	0.5	2.0
0.10	0	0	3.13	0.6	2.0
0.20	20**	1.2**	3.32	0.6	2.9
0.40	34*	3.0	3.62	0.7	3.0
0.60	36*	4.0	4.22	0.8	3.2
0.80	42	5.0	4.56	0.9	3.6
1.00	56	6.2*	5.19	1.2	5.0
2.00	64	6.9*	5.19	1.2	5.0
4.00	68	8.7**	5.14	1.3	5.2
6.00	74	12.4**	5.13	1.4	5.0
Unsterilized, uninoculated	58	4.2	4.98	0.8	4.0

*, ** Significantly different from the unsterilized, uninoculated at the 95 % and 99 % confidence levels, respectively. Zero values are highly significant.

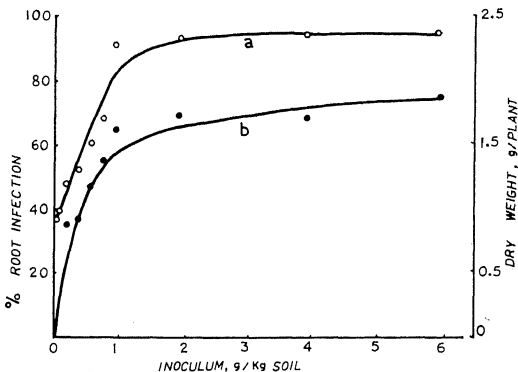


Figure 1. Impact of VA mycorrhizal inoculum (dried root powder) levels on **a.** plant dry matter production and **b.** per cent root infection in groundnut, after 100 days of plant growth.

Inoculum	Days after sowing	VA mycorrhiza		Nodules		Plant analysis		
		Root length infected (%)	Vesicles or spores cm ⁻¹ root	Number plant ⁻¹	Dry wt plant ⁻¹ (mg)	Average dry matter (g/plant)	N (%)	P (%)
(a) cv TMV-2								
Uninoculated	60	0	0	0	0	0.66	3.98	0.6
	90	0	0	0	0	1.05	4.10	0.8
<i>Glomus mosseae</i>	60	32	2.6	0	0	0.93	4.48	1.1
	90	44	3.9	0	0	1.32	5.73	1.7
<i>Rhizobium</i> sp	60	0	0	23.0	5.0	1.04	5.15	1.1
	90	0	0	25.0	6.0	1.63	5.93	1.5
<i>Rhizobium</i> sp + <i>Glomus mosseae</i>	60	48*	4.2*	41.5	10.0	2.60	5.68	1.8
	90	60*	5.8*	57.0	12.0	3.41	6.29	2.7
(b) cv Robut 33-1								
Uninoculated	60	0	0	0	0	1.02	4.37	0.7
	90	0	0	0	0	1.49	5.19	1.0
<i>Glomus mosseae</i>	60	32	2.9	0	0	1.29	5.10	1.4
	90	48	4.2	0	0	2.10	5.57	1.9
<i>Rhizobium</i> sp	60	0	0	44.0	20.0	1.46	5.62	1.4
	90	0	0	42.0	20.0	2.25	6.24	1.7
<i>Rhizobium</i> sp + <i>Glomus mosseae</i>	60	64*	5.8*	85.0	37.5	3.15	6.20	2.6
	90	72*	6.6*	88.0	36.0	3.94	6.73	3.3

* Significantly different ($P < 0.05$) from the corresponding values with *G. mosseae* inoculation alone.

with *Rhizobium* sp nodulated well. The number, size and dry weight of nodules in the plants inoculated with *G. mosseae* plus *Rhizobium* were greater than in the plants inoculated with *Rhizobium* sp alone, at 60 and 90 days after inoculation. Thus, in combined inoculations there were 57 nodules per plant as against 25 nodules when *Rhizobium* sp alone was used as the inoculum, at 90 days of plant growth.

The uninoculated control plants and the plants inoculated with only *Rhizobium* sp did not show any mycorrhizal infection. The plants receiving the combined inoculum of *Rhizobium* sp and *G. mosseae* gave significantly ($P < 0.05$) high levels of colonization of roots and sporulation by the mycorrhizal fungus, when compared with the plants receiving only the mycorrhizal fungus. The dry weight and the per cent of nitrogen and phosphorus were higher in plants raised with *Rhizobium* sp and *G. mosseae* than in separate inoculations or in uninoculated plants. This increase was most pronounced when the plants were 90 days old.

Almost similar results were obtained with cv Robut 33-1 and the effects of combined inoculum were even greater in this variety when compared with TMV-2 cultivar (table 2).

4. Discussion

Although the relationship between the plant and the fungus in VAM formation is symbiotic, the primary process in the establishment of the fungus in roots is infection.

inoculum potential of *G. mosseae* in the present study was assessed by using different quantities of powdered roots from plants showing heavy infection. The results clearly show that 1 g dried root inoculum kg⁻¹ soil was optimal for mycorrhizal formation in groundnut by *G. mosseae* which almost equalled the level of infection in unsterilized soil. Furthermore, the data indicate that dry matter production was closely associated with infection, both reaching a plateau beyond 1.0 g inoculum kg⁻¹ soil. Likewise, Carling *et al* (1979) reported an increase in the infection units (entry points) in young soybean seedlings with increasing quantities of *Glomus fasciculatus* inoculum, until a maximum was reached. Smith and Walker (1981) while presenting a mathematical model of the infection also established a similar relationship between inoculum and infection of roots of *Trifolium subterraneum* by VA mycorrhizal fungi.

The present investigation also brings out clearly that VA mycorrhiza greatly enhanced nodulation in groundnut plants inoculated with *Rhizobium* sp. The relatively lower response of plants at 60 days of inoculation with *Rhizobium* sp or *Rhizobium* sp + *G. mosseae* or *G. mosseae* only when compared to 90 days suggests that the crop derived greater benefit from nitrogen fixed as well as from P taken up in later stages of plant growth. These findings are in close agreement with those of Mosse (1977) on *Stylosanthes* sp in pot culture experiments using unsterilized soil and those reported by Schenck and Hinson (1973) for dually infected soybeans. Mosse (1977) also regarded that the principal effect of mycorrhizas on nodulation is phosphate-mediated.

References

- Asimi S V, Gianinazzi P and Gianinazzi S 1980 Influence of increasing soil phosphorus levels on interactions between VA mycorrhiza and *Rhizobium* in soybeans; *Can. J. Bot.* **58** 2200–2205
- Bagyaraj D J, Manjunath A and Patil R B 1979 Interaction between a VAM and *Rhizobium* and their effects on soybean in the field; *New Phytol.* **82** 141–145
- Carling D E, Brown H F and Brown R A 1979 Colonization rates and growth responses of soybean plants infected by vesicular-arbuscular mycorrhizal fungi; *Can. J. Bot.* **57** 1769–1772
- Crush J R 1974 Plant growth responses to vesicular-arbuscular mycorrhiza. VII. Growth and nodulation of some herbage legumes; *New Phytol.* **73** 743–749
- Daft M J and El-Giahmi A A 1976 Studies on nodulated and mycorrhizal peanuts; *Ann. Appl. Biol.* **83** 273–276
- Fogg D N and Wilkinson N T 1958 The colorimetric determination of phosphorus; *Analyst (London)* **83** 406–414
- Jackson M L 1967 *Soil chemical analysis* (New Delhi: Prentice Hall)
- Mosse B 1977 Plant growth responses to VA mycorrhiza. X. Responses of *Stylosanthes* and maize to inoculation in unsterile soils; *New Phytol.* **78** 277–288
- Mosse B, Powell C L and Hayman D S 1976 Plant growth responses to VAM. IX. Interactions between VAM, rock phosphate and symbiotic nitrogen fixation; *New Phytol.* **76** 331–342
- Nicolson T H 1959 Mycorrhiza in the Gramineae. I. Vesicular-arbuscular endophytes, with special reference to the external phase; *Trans. Br. Mycol. Soc.* **42** 421–438
- Parvathi K, Venkateswarlu K and Rao A S 1985 Effects of pesticides on development of *Glomus mosseae* in groundnut; *Trans. Br. Mycol. Soc.* **84** 29–33
- Phillips J M and Hayman D S 1970 Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection; *Trans. Br. Mycol. Soc.* **55** 158–161
- Rao A S and Parvathi K 1982 Development of vesicular-arbuscular mycorrhiza in groundnut and other hosts; *Plant Soil* **66** 133–137

- Schenck N C and Hinson K 1973 Response of nodulating and non-nodulating soybeans; *Agron. J.* **65** 849-850
- Smith S E and Daft H J 1977 Interactions between growth, phosphate content and nitrogen fixation in mycorrhizal and non-mycorrhizal *Medicago sativa*; *Aust. J. Plant Physiol.* **4** 403-413
- Smith S E and Walker N A 1981 A quantitative study of mycorrhizal infection in *Trifolium*: Separate determination of the rates of infection and of mycelial growth; *New Phytol.* **89** 225-240

Effect of nitrogen fertilizer on biomass and growth behaviour of two range grasses

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Abstract. The effect of four levels of nitrogen (0, 20, 40 and 80 ppm) on the growth behaviour of two range grasses (i.e. *Lolium perenne* and *Poa pratensis*) was studied. The application of different doses of fertilizer increased the shoot and root biomass, the former to a greater degree. The inducing effect of increasing levels of nitrogen was more pronounced on the aerial parts than on the underground parts as indicated by decreasing root:shoot ratio. Different growth parameters such as relative growth rate, specific and total leaf area also increased significantly with increasing levels of nitrogen.

Keywords. Nitrogen fertilizer; grasses; biomass.

1. Introduction

Nitrogen has considerable effect on photosynthesis and growth of plants. Photosynthetic output may increase upon nitrogen application (Murata 1969). The increase in dry matter yield may be due to increased photosynthetic surface (Luxmoore and Millington 1971). N application may also affect carbohydrate reserves unfavourably in some cases (Priyanishnikov 1951). The present study was undertaken to examine the influence of different levels of N on dry matter yield of *Lolium perenne* L (perennial rye-grass) and *Poa pratensis* L (Kentucky blue-grass). These species are components of native sward.

2. Methods of study

Tillers of *L. perenne* and *P. pratensis* were collected in June 1982 from a native sward at Nainital. Tillers of uniform size were transplanted into polyethylene pots filled with known amount of garden soil and grown in a glass house at Nainital (29°24' N lat, 79°28' E long, 2050 m altitude) between June and August 1982. One tiller was grown per pot. All pots were irrigated to field capacity to bring the soil water to a uniform level. For each species a total of 60 pots were used. The pots were arranged into four sets of 15 pots each. One set was treated as control (without N application). The soil in the remaining three sets was amended by applying N exogenously at 20, 40 and 80 ppm. Exogenous N was applied in the form of ammonium nitrate (NH_4NO_3) and therefore all of it was available to the plant. The nitrogen content of the soil was 0.12%; however the available N would only be a small fraction of the total N. To begin with N was applied only once. Pots were irrigated every 4–5 days. First sampling was done before N was applied. Three plants from each treatment were sampled at random for harvest at zero time (first sampling) and subsequently at 15-day interval for about 75 days. The

leaf area was measured using a planimeter and the leaf samples were then used to determine chlorophyll. These were cut into small pieces, homogenized and extracted in a mixture of acetone and water (4:1 v/v). The pigment concentration was determined using a spectrophotometer following the methods of Arnon (1949) for chlorophyll a, b and total chlorophyll.

The following expressions based on Evans (1972) were used to calculate different growth parameters.

$$\text{Relative growth rate (RGR)} = \frac{\log W_2 - \log W_1}{T_2 - T_1},$$

$$\text{Specific leaf area (SLA)} = \frac{A}{Lw_1},$$

$$\text{Total leaf area (TLA)} = \text{SLA} \times Lw,$$

$$\text{Leaf weight ratio (LWR)} = \frac{Lw}{W},$$

$$\text{Leaf area ratio (LAR)} = \text{SLA} \times \text{LWR},$$

$$\text{Net assimilation rate (NAR)} = \frac{W_2 - W_1}{Lw_x (T_2 - T_1)},$$

where W_1 and W_2 are dry weights at time T_1 and T_2 , T_1 and T_2 are number of days in sampling interval, A is the leaf area (cm^2), Lw_1 is the weight of leaf samples taken for leaf area, Lw is the dry weight of leaves, w is the total plant dry weight and Lw_x is the leaf dry weight at T_1 . All weights are expressed in grams. A two-way analysis of variance was used to analyse the differences in shoot, root and total biomass due to sampling dates and fertilizer treatments.

3. Results

Root, shoot and total biomass of *L. perenne* and *P. pratensis* increased with time attaining the maximum value at final harvest. Their root, shoot and total biomass varied significantly with treatments (i.e. N amendment) ($p < 0.05$) and sampling dates ($p < 0.01$). Both the species have an appreciable response to nitrogen application. Their dry matter yield increased with increasing levels of nitrogen fertilizer. The overall performance of fertilizer-treated plants of *L. perenne* was much superior compared to *P. pratensis*. In both the species the increase in total biomass was maximum for plants treated with 80 ppm N, values increasing from 0.24 g plant⁻¹ (initial value) to 5.09 g plant⁻¹ (final value) and 0.29 g plant⁻¹ to 5.96 g plant⁻¹ in *P. pratensis* and *L. perenne* respectively. On the contrary, the values obtained for controlled plants were considerably low (increase in value was 0.24 g plant⁻¹ to 1.92 g plant⁻¹ in *P. pratensis* and 0.25 g plant⁻¹ to 2.21 g plant⁻¹ in *L. perenne*). Table 1 indicates the degree to which N fertilizer affected total as well as biomass of different organs as the final harvest. The favourable effect of N fertilizer on shoot biomass was comparatively greater in

Table 1. Effect of N fertilizer on different growth parameters of two grass species at final harvest.

Growth parameters	<i>L. perenne</i>				<i>P. pratensis</i>			
	0 ppm	20 ppm	40 ppm	80 ppm	0 ppm	20 ppm	40 ppm	80 ppm
Total plant biomass (g plant ⁻¹)	2.21	3.36	3.94	5.96	1.92	2.95	3.99	5.09
Shoot biomass (g plant ⁻¹)	1.40	2.08	2.61	4.07	1.22	1.88	2.47	3.69
Root biomass (g plant ⁻¹)	0.81	1.28	1.47	1.89	0.70	1.07	1.38	1.43
Tiller number (No. plant ⁻¹)	10.50	20.00	27.50	31.00	11.50	20.20	22.40	26.00
Tiller height (cm)	25.10	36.70	41.30	45.40	22.80	28.60	30.30	35.20
Total leaf area (cm ² plant ⁻¹)	385.9	814.7	1242.1	2791.6	288.0	565.4	583.7	1890.0

magnitude in *L. perenne* compared to *P. pratensis*. At the final harvest, the plants treated with 80 ppm nitrogen exhibited maximum increase in shoot dry matter (3.56 g plant⁻¹ increase in *P. pratensis* and 3.88 g plant⁻¹ in *L. perenne*) followed by 40 ppm (2.34 g plant⁻¹ increase in *P. pratensis* and 2.42 g plant⁻¹ in *L. perenne*) and 20 ppm (1.75 g plant⁻¹ increase in *P. pratensis* and 1.89 g plant⁻¹ in *L. perenne*). Compared to these, accumulation of shoot dry matter in controlled plants was very low (1.09 g plant⁻¹ in *P. pratensis* and 1.21 g plant⁻¹ in *L. perenne*) (values in parenthesis represent the net change from the first to the last value).

Table 1 shows that the root biomass values on controlled plants were always lower than on fertilizer-treated plants. The values were again maximum with *L. perenne*. The root biomass was comparatively less affected by N application compared to shoot biomass. Application of nitrogen fertilizer increased the root biomass in the following order: 80 ppm (1.79 g plant⁻¹ in *L. perenne* and 1.33 g plant⁻¹ in *P. pratensis*), 40 ppm (1.37 g plant⁻¹ in *L. perenne* and 1.28 g plant⁻¹ in *P. pratensis*), 20 ppm (1.18 g plant⁻¹ in *L. perenne* and 0.97 g plant⁻¹ in *P. pratensis*) and controlled plants (0.71 g plant⁻¹ in *L. perenne* and 0.60 g plant⁻¹ in *P. pratensis*). The tiller number and plant height of both the species also increased in the same order (table 1).

In both the species root:shoot ratio of plants treated with N fertilizer was generally lower than that of controlled plants during most of the experimental period. At the final harvest root:shoot ratio in *L. perenne* was 0.47, 0.56, 0.61 for plants treated with 80, 40 and 20 ppm N respectively and 0.58 in controlled plants. The corresponding values in *P. pratensis* were 0.39, 0.56, 0.56 and 0.57.

Mean RGR of whole plant as well as of shoots (R_s GR) and roots (R_r GR) were higher for plants treated with nitrogen fertilizer in both the species (table 2). RGR fluctuated considerably with time. Under each treatment the rate declined towards the end of the experimental period. Like RGR chlorophyll concentration also declined towards the end

Exogenously applied N (ppm)	<i>L. perenne</i>			<i>P. pratensis</i>		
	R _S GR	R _R GR	ULR	R _S GR	R _R GR	ULR
0	0.013	0.035	0.089	0.012	0.028	0.087
20	0.017	0.042	0.121	0.015	0.034	0.120
40	0.025	0.052	0.151	0.019	0.038	0.155
80	0.029	0.058	0.151	0.021	0.045	0.186

All values are expressed as $\text{g g}^{-1} \text{ day}^{-1}$

Table 3. Effect of N fertilizer on different parameters (an average value for the entire experimental period) of two grass species

Growth parameters	<i>L. perenne</i>				<i>P. pratensis</i>			
	0 ppm	20 ppm	40 ppm	80 ppm	0 ppm	20 ppm	40 ppm	80 ppm
Chlorophyll a (mg g^{-1})	7.2	8.0	10.2	11.7	3.8	5.0	5.6	7.0
Chlorophyll b (mg g^{-1})	3.5	4.4	5.6	6.7	2.7	3.2	3.5	4.5
Total chlorophyll (mg g^{-1})	10.7	12.4	15.8	18.8	6.5	8.2	9.1	11.5
Specific leaf area ($\text{cm}^2 \text{g}^{-1}$)	435.6	460.6	551.2	668.2	302.0	397.2	360.8	525.6
Leaf weight ratio	0.46	0.44	0.43	0.46	0.48	0.48	0.47	0.49
Leaf area ratio	205.5	196.8	237.8	313.2	153.6	190.4	168.2	264.3

found in higher concentration than chlorophyll b throughout the experimental period. TLA increased with time under each treatment. At the final harvest the highest TLA was found in plants treated with 80 ppm N. TLA increased as 1%, 49.1%, 50.7% and 84.8% in *P. pratensis* and 52.6%, 68.9% and 86.7% in *L. perenne* at 0, 20, 40 and 80 ppm respectively.

SLA revealed greatest value for plants receiving 80 ppm N followed by those receiving 40 and 20 ppm and controlled plants (table 3). Application of N did not exhibit any consistent effect on LWR. In contrast, LAR increased markedly with increasing levels of N. Unit leaf rate (ULR) also showed a fluctuating trend with time. LAR and ULR also declined towards the final harvest.

4. Discussion

Application of different doses of N significantly increased the total biomass and biomass of different organs in two grasses. The maximum increase was obtained by 80 ppm N which was followed by 40 and 20 ppm N application. These results agree with

was associated with an increase in tiller number and tiller height. According to Langer (1963) and Luxmoore and Millington (1971) tillering is a major factor associated with the leaf expansion and dry matter production. Adequate supply of N accelerates the rate of photosynthesis and increases the number of tillers. N amendment increased shoot growth more than the root growth. Several workers reported a decrease in root: shoot ratio of plants treated with N fertilizer (Hocking 1972; Reddy *et al* 1976). An increase in the supply of nitrogen to roots may decrease both root and shoot growth, the later to a greater extent (Ettar 1971; Hocking 1972; Chauhan and Khosla 1978). Mathews (1972) mentioned that increased doses of fertilizer increases the shoot weight without a corresponding increase in root weight. Luxmoore (1971) showed that reduction in N supply resulted in an increase in root:shoot ratio.

Increasing dose of N also stimulated the concentration of chlorophyll in leaves. Maximum concentration was recorded in plants receiving 80 ppm N at each harvest. Further concentration of chlorophyll declined with time in both grasses. Bokhari (1976) also observed a decline in pigment concentration with time in western wheat grass and blue grama.

N application proportionately increased TLA in both species. The increase in TLA was attributed by Langer (1963) and Luxmoore (1971) to tillering which increases plant's biomass and expansion of leaves.

In the present investigation N fertilizer increased the number of tillers, total dry weight and TLA. Like chlorophyll concentration, RGR, LAR and ULR also increased by N application. 80 ppm N was found most effective followed by 40 and 20 ppm N. Similar results were obtained by Kumar (1976) and Thorne (1960).

Our results show that N application increased the production of both grasses. However, *L. perenne* performed better compared to *P. pratensis*. N application stimulated the production of more leaves having a greater photosynthetic potential as indicated by greater concentration of chlorophyll and higher unit leaf rate.

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References

- Arnon D I 1949 Copper enzymes in isolated chloroplasts; *Plant Physiol.* **24** 1-15
- Chauhan P S and Khosla P K 1978 Effect of application of fertilizer on the growth of CHLL Seedling (*Pinus roxburghii* Sargent); *Indian J. For.* **14**
- Ettar H M 1971 Nitrogen and phosphorus requirements during the early growth of white spruce seedlings; *Can. J. Plant Sci.* **51** 61-63
- Evans G C 1972 *The quantitative analysis of plant growth* (Oxford, London, Edinburgh, Melbourne: Blackwells)
- Hocking D 1972 Current rearing knowledge proceedings of a workshop on container planting in Canada; Information report DPC-X-2, Ontario, 48-66
- Kumar R 1976 Dry matter production and growth rates of three arid zone grasses in culture; *Comp. Physiol. Ecol.* **1** 23-26
- Kumar R, Dabaghao P M, Shankarnarayan K A and Rai P 1980 Effect of different levels of N and P on the forage potential of *Cenchrus setigerus* Vahl; *Indian J. Agric. Sci.* **50** 488-491
- Langer R H M 1963 Tillering in herbage grasses; *Herbage Abstr.* **33** 141-148

- Luxmoore R L and Millington R J 1971 Growth of perennial rye-grass (*Lolium perenne* L.) in relation to water, N and light intensity *Plant Soil* **34** 269-281
- Mannejit L T and Shaw N H 1972 Nitrogen fertilizer responses of a *Heteropogon contortus* and *Paspalum plicatulum* pasture in relation to rainfall in central coastal queensland; *Aust. J. Exp. Agric. Animal Husbandry* **12** 28-35
- Mathews R G 1972 A look of shoots and roots in the nursery; *Proc. Workshop in container planting in Canada*; Information report DPC-X-2, Ontario, 72-76
- Murata Y 1969 Physiological responses to nitrogen in plants In *Physiological aspects of crops yield* (eds) J D Eastin, T A Haskins, C 4. Sullivan and C H M Van Bavel (Wisconsin Am. Soc. Agron.) 235-259
- Prianishnikov D N 1951 Nitrogen in the life of plants. Translated by S A Wilde. Kramer Business Service, Inc., Madison, Wisconsin.
- Reddy R, Khan M A and Dabryol 1976 Effect of compost and mineral fertilizer on development of Mycorrhiza in Chir pine (*Pinus roxburghii* Sargent) and Patula pine (*P. patula* Schlecht & Cham) *Ind. For.* **102** 463-471
- Thorne G N 1960 Variation with age in out assimilation rate and other growth attributes of sugar beet, potato and barley in a controlled environment; *Ann. Bot.* **24** 356-371

Fractional coagulation of proteins from alfalfa leaf juice by use of alum

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Abstract. A process is described to fractionate the expressed alfalfa juice into green and white protein-rich fractions using alum. Fractional coagulation using heat yielded more white product than when alum was used. This was attributed to more protein precipitated in green fraction when alum was used. Protein precipitating ability of alum was seen to be as good as that of trichloroacetic acid. The cost of separation of green and white fractions using alum is likely to be low since the technique is simple and capital saving.

Keywords. Protein from leaves; alum; fractional precipitation; leaf proteins.

1. Introduction

The acceptance of plant protein concentrate obtained from freshly expressed alfalfa juice as human food has been retarded by objections associated with its green colour and grassy flavour. Recent technological advances have, however, made it possible to obtain buff or cream-coloured bland products from alfalfa juice (Huang *et al* 1971; Knuckles *et al* 1975; Anelli *et al* 1977; Bray and Humphris 1979; Pirie 1978). Protein in juice is in the form of a hydrophilic sol and the effectiveness of ions in salting out hydrophilic sols is given in lyotropic series (Levitt 1954). In this series K^+ ranks third and SO_4^{2-} ions ranks first. Due to the presence of both K^+ and SO_4^{2-} ions in alum ($K_2SO_4 \cdot Al_2(SO_4)_3 \cdot 24 H_2O$), it was considered a precipitant worth investigating to obtain a white bland protein concentrate. Alum is readily available commercially and is routinely used in Indian households for purifying drinking water.

2. Materials and methods

The juice from alfalfa (*Medicago sativa* L) harvested at the early flowering stage was expressed using the International Biological Programme equipment (Pirie 1971). Alum was used for precipitating the protein-rich fractions.

2.1 Fractional coagulation of proteins in the juice

2.1a Separation of green chloroplastic fraction: The juice was slowly added, accompanied by stirring, to a pan holding water maintained at $55^\circ C$ to precipitate a green coagulum which was separated from the brown supernatant by centrifugation. Juice was allowed to stand for different periods of time and then centrifuged. The time for separation of green coagulum from the clear brown supernatant was noted. Ten ml of 10% (w/v) alum solution were added to 100 ml of fresh juice which was then

Sample	Crude protein (%) (N × 6)	Ether extract (%)	Ash (%)	Insoluble ash (%)
1	50	4.1	12.3	1.8
2	46	4.5	6.2	5.0
3	45	5.3	10.4	4.8

Supernatants were separated from green fraction by cloth filtration under gravity.

2.1b Separation of white cytoplasmic fraction from supernatant: The supernatant was slowly added, accompanied by stirring, to a pan holding water maintained at 90°C to precipitate the white fraction. To 100 ml of supernatant 5 ml of (10% w/v) alum solution were added and a white precipitate was obtained. The pH of supernatant was adjusted to pH 4.0 using dilute HCL to precipitate the white fraction. An equal volume of cold 10% (w/v) TCA was added to the supernatant to precipitate the white fraction.

White and green fractions thus obtained were thoroughly washed with distilled water and centrifuged. The process was repeated until the supernatant was colourless. The sediment was dried at 60°C.

All centrifugations were conducted at 5000 r.p.m. for 10 min. Pilot scale precipitation of the chloroplastic fraction was achieved by adding 100 ml of 10% (w/v) alum solution to 1000 ml of freshly expressed juice. The chloroplastic fraction was separated by filtration using thick cotton cloth under gravity. The supernatant obtained was treated with alum solution to obtain white cytoplasmic fraction. The proximate analysis of the cytoplasmic fraction thus obtained is given in table 1.

Total ash, acid soluble and insoluble ash and ether extract were estimated in the white fraction according to the AOAC (1965) methods. Nitrogen was determined by the standard microkjeldahl procedure (AOAC 1965) and crude protein was calculated as $N \times 6.0$ (Pirie 1955).

3. Results and discussion

When the juice was left to stand (see §2.1a) it took about 4.5 hr to obtain the green coagulum. The yields of green and white fractions obtained by treatment with alum ranged from 30 to 39 g and 4.20 to 7.4 g/litre of fresh juice respectively. The yields of both green and white fractions were found to depend upon the dry matter content of juice, which varied from 7 to 11%.

When the juice was differentially heat-coagulated to obtain green and white fractions, the yield of the latter fraction was 9.45 g/litre of fresh juice. The same batch of juice was treated with alum solution to obtain green fraction. The white fraction was then collected by treating the supernatant with alum solution and adjustment to pH 4.0. The yields of white fraction obtained in the experiment were 5.3 and 4.81 g/litre of fresh juice respectively. This result raised the question whether the lower yield of white fraction using alum in comparison to that obtained by heat was due to the imperfect coagulation of proteins present in the supernatant.

fraction was separated from the supernatant by alum treatment, adjustment to pH 4.0 and heating to 90°C. The yields of white fractions in this experiment were 3.85, 3.60 and 3.50 g/litre respectively. The second batch was heat-treated to obtain the green and white fractions. The yield of white fraction was 5.98 g/litre of juice. These results indicate that the precipitating ability of the alum was almost equal to that of the heat treatment. The enhanced yield of white fraction in differential heat coagulation must therefore be due to the lesser amount of protein separated in the green fraction than with the alum treatment.

The yield of dry matter of white fraction by TCA precipitation and that resulting from the addition of 5 ml of 10% (w/v) alum solution was found to be equal, indicating that the protein precipitating ability of alum was as good as that of TCA. These results show that alum is a convenient and efficient protein-coagulating agent.

The proximate composition of white protein obtained on a pilot scale using the technique of alum precipitation is given in table 1. The three products are from three different batches of juice. The constituents which remain as balance are carbohydrates which include crude fibre and nitrogen-free extract. Crude fibre is usually below 1%.

It is considered that to obtain 1 kg of white protein the alum required will cost 8c. The procedure does not need elaborate controls in contrast to the differential heat coagulation procedure. Centrifugation used in the present laboratory investigation could be replaced by simple gravity filtration through thick cotton cloth for bulk handling. Thus this could be a technically simple process worth investigating further with emphasis on finding ways to check the amounts of protein precipitating in the green fraction.

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References

- AOAC 1965 *Official method of analysis* (Washington: Association of Official Agricultural Chemists) 10th ed.
- Anelli G, Fiorentini R, Massignan L and Galoppini C 1977 The poly-protein process: A new method for obtaining leaf protein concentrates; *J. Food Sci.* **42** 1401
- Bray W and Humphris C 1979 Preparation of white leaf protein concentrate using a polyanionic flocculant; *J. Sci. Fd Agric.* **30** 171
- Huang K H, Tao M C, Boulet M, Riel R R, Julien J P and Brisson G J 1971 A process for preparation of leaf protein concentrates based on the treatment of leaf juices with polar solvents; *Can. Inst. Fd Technol.* **4** 85
- Knuckles B E, de Fremery D, Bickoff E M and Kohler G O 1975 Soluble protein from alfalfa juice by membrane filtration; *J. Agric. Fd Chem.* **23** 209
- Levitt J 1954 *Plant physiology* (New York: Prentice Hall)
- Pirie N W 1955 *Modern methods of plant analysis*. (eds) K Paech and M V Tracey, (Berlin: Springer)
- Pirie N W 1971 *Leaf protein: its agronomy, preparation, quality and use* (Oxford: Blackwells) p. 53
- Pirie N W 1978 *Separation, purification, composition and fractionation in leaf protein and other aspects of fodder fractionation*. (Cambridge: University Press) p. 47

The genus *Ramaria* in the Eastern Himalayas: Subgenera *Ramaria*, *Echinoramaria* and *Lentoramaria*

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Abstract. In this paper an account of 12 taxa of the genus *Ramaria* (Fr) Bonorden collected from various localities in the eastern Himalayas and adjoining hills is given. Of the taxa included *R. rubella* var *himalaica* is described as new; *R. rubrievanescens*, *R. rubripermanens*, *R. botrytis*, *R. straxeri* and *R. flaccida* var *longiramosa* are now records for the Himalayas; while *R. clarobrunnea*, *R. flaccida*, *R. pusilla*, *R. concolor* and *R. apiculata* are the first records of their occurrence in the eastern Himalayas.

Keywords. *Ramaria*; taxonomy; eastern Himalayas.

Introduction

Fungal forays conducted by the authors during the years 1978–1981 revealed luxuriant growth of clavarioid fungi in the eastern Himalayas. Most of the collections made pertained to the species of genus *Ramaria*. This paper gives an illustrated account of 12 taxa of the genus, of which one is described as new, five are new records for the Himalayas and five are the first records of their occurrence in the eastern Himalayas.

Ramaria is the largest genus of clavarioid fungi and the modern trend is to treat it under several subgenera. The species described in this paper belong to the subgenera *Ramaria*, *Echinoramaria* and *Lentoramaria*. The materials of all the taxa have been deposited at the Herbarium, Department of Botany, Panjab University, Chandigarh, (PAN) and at some noted foreign herbaria as indicated. The abbreviations used for herbaria follow Holmgren and Keuken (1974) and the colour standards are according to Kornerup and Wanscher (1967).

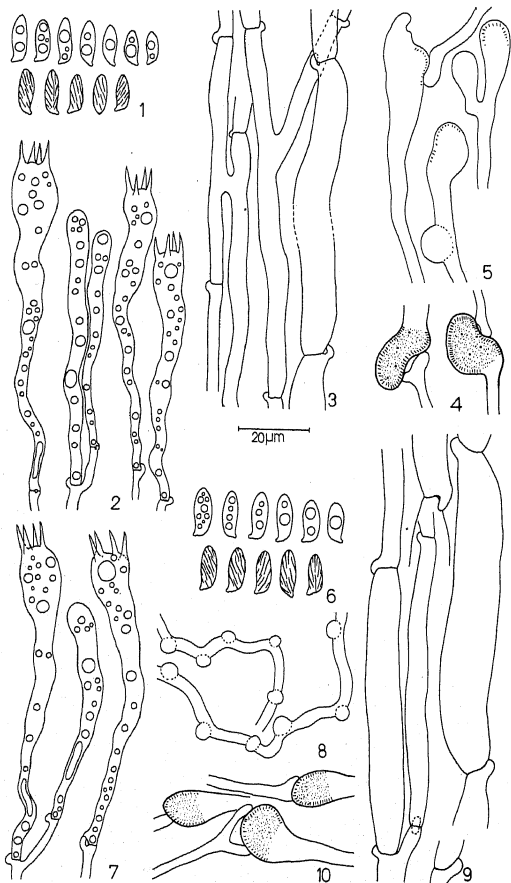
Subgenus: *Ramaria*

1. *Ramaria rubrievanescens* Marr & Stuntz, Bibliotheca mycol.

8: 41. 1973. (figures 1–5)

Fruit-bodies up to 15 × 9 cm, massive, fleshy, gregarious or scattered, cream-coloured, turning brown to brownish red on bruising; trunk absent; branching polychotomous throughout, lower branches thick, becoming narrower upward; axils lunate; tips subacute to obtuse, mostly in pairs, pink but fading later on; flesh white; taste and smell not distinctive.

Hyphal system monomitic; hyphae upto 11 (– 13.5) μ m wide, clamped, thin-walled, cyanophilous; ampullaeform swellings prominently ornamented; gloeoplerous hyphae abundant, up to 5 μ m wide, simple to sparsely branched, inflated near the apices, thin-walled, cyanophilous. Basidia 70–91 × 9.5–11 μ m, clavate, clamped,



Figures 1–10. *R. rubrievanescens*. 1. Basidiospores. 2. Basidia. 3. Context-hyphae.

guttulate, 4-spored; sterigmata up to $7\text{ }\mu\text{m}$ long. Basidiospores $9\text{--}11(-12.5) \times 3.5\text{--}4.5(-5)\text{ }\mu\text{m}$, cylindric-ellipsoid, uni- to multiguttulate; wall thin to slightly thickened, cyanophilous, striated; apiculus up to $1\text{ }\mu\text{m}$ long.

Specimens examined: R. M. Sharda 22279 (PAN), on soil under mixed forest, Nawephu, Thimphu, Bhutan, September 26, 1980; R. M. Sharda 22322 (PAN), on soil under mixed forest, Begana, Thimphu, Bhutan, August 2, 1981; R. M. Sharda 22348 (PAN), on soil under broad-leaved forest, Chankaphug, Thimphu, Bhutan, August 9, 1981.

The eastern Himalayan collections resemble closely with those of *Dalhousie* in the north-western Himalayas made by Khurana (1977). This species is closely allied to *R. rubripermanens* but differs in having cream colour of the fruit-bodies turning brown to brownish red on bruising and pink tips fading later on.

2.2 *Ramaria rubripermanens* Marr & Stuntz, *Bibliotheca mycol.*

38: 43. 1973 (figures 6–10)

Fruit-bodies up to $13 \times 14\text{ cm}$, massive, fleshy-watery, gregarious or scattered; pinkish white, unchanging on bruising; trunk up to $4 \times 3\text{ cm}$, white, branches polychotomous, up to 1.5 cm wide at the base, becoming thinner and longer above; axils wide open; tips acute to subacute, in pairs or clustered, pink, not fading later on; flesh concolorous with the fruit-body surface; taste and smell not distinctive.

Hyphal system monomitic; hyphae up to $12.5(-16)\text{ }\mu\text{m}$ wide, clamped, thin to slightly thick-walled, acyanophilous; ampullaeform swellings prominently ornamented; gloeoplerous hyphae rare, up to $3.5\text{ }\mu\text{m}$ wide, simple or branched, with abundant localized swellings imparting beaded appearance, thin-walled, cyanophilous. Basidia up to $80 \times 10.5\text{ }\mu\text{m}$, clavate, guttulate, clamped, 4-spored; sterigmata up to $7\text{ }\mu\text{m}$ long. Basidiospores $10.5\text{--}12(-13) \times 4\text{--}4.5(-5)\text{ }\mu\text{m}$, ellipsoid, uni- to multiguttulate; wall thin to slightly thickened, cyanophilous, spirally striated; apiculus up to $0.8\text{ }\mu\text{m}$ long.

Specimens examined: R. M. Sharda 22280 (PAN), on soil under mixed forest, Nawephu, Thimphu, Bhutan, September 26, 1980; R. M. Sharda 22319 (PAN), on soil under mixed forest, Begana, Thimphu, Bhutan, August 2, 1981; R. M. Sharda 22347 (PAN), on soil under mixed forest, Chankaphug, Thimphu, Bhutan, August 9, 1981.

Khurana (1977) made a number of collections of this species from *Dalhousie* and *Simla* hills in the north-western Himalayas which closely resemble the eastern Himalayan collections made by us. The species is marked by pinkish white fruit-bodies having clearly pink tips not fading later on and non-bruising nature.

2.3 *Ramaria botrytis* (Pers.: Fr.) Ricken, *Vademecum*. 253. 1918 (figures 11–13)

Fruit-bodies up to $12 \times 13.5\text{ cm}$, nearly as broad as tall, massive, fleshy-fibrous, gregarious or scattered closely, rarely in groups of 2 or 3, light yellow at the base and flesh-coloured to reddish or vinous upward, unchanging on bruising; trunk indistinct to distinct, up to $2.5 \times 2\text{ cm}$, somewhat stubby, dull yellow; branches polychotomous, lower branches up to 1 cm thick, stout, becoming shorter and congested upward, smooth or faintly rugulose; axils narrow; tips subacute to obtuse, minute, pluridigitate, pale red to pastel red or vinous; flesh white; taste not distinctive; smell pleasant, edible.

acyanophilous; ampullaeform swellings conspicuously ornamented; gloeoplerous hyphae not observed. Basidia up to $70 \times 10.5 \mu\text{m}$ clavate, guttulate, clamped, 4-spored; sterigmata up to $7 \mu\text{m}$ long. Basidiospores $12-15 (-16.5) \times 4-4.5 (-5) \mu\text{m}$, cylindric-ellipsoid, uni- to multiguttulate; wall thin, cyanophilous, striated; apiculus up to $1.2 \mu\text{m}$ long.

Specimens examined: R. M. Sharda 22373 (PAN), on soil under mixed forest, Chankaphug, Thimphu, Bhutan, August 16, 1981; R. M. Sharda 22467 (PAN), on soil under broad-leaved forest, Shergaon, West Kameng, Arunachal Pradesh, September 8, 1981; R. M. Sharda 22470 (PAN), on soil under mixed woods, 15 km (Rupa-Shergaon road), West Kameng, Arunachal Pradesh, September 9, 1981.

All the eastern Himalayan collections are quite typical of the species and agree well with the description given by Corner (1950, 1970) and Marr and Stuntz (1973).

2.4 *Ramaria strasseri* (Bres) Corner, Ann. Bot. Mem. 1: 622. 1950 (figures 14-18)

Fruit-bodies up to $17 \times 10 \text{ cm}$, massive, fleshy, occurring singly, light yellow to greyish orange at the base, pale yellow to tan above, unchanging on bruising; trunk up to $7.5 \times 3.5 \text{ cm}$, massive, thick, white; branches polychotomous below and dichotomous above, basal branches up to 1 cm thick, becoming thinner and elongated upward; axils U-shaped; tips subacute to obtuse, bifid to multifid, light brown with vinaceous cast, turning dark-brown after drying; flesh white, soft, fibrous; taste bitter; smell not distinctive.

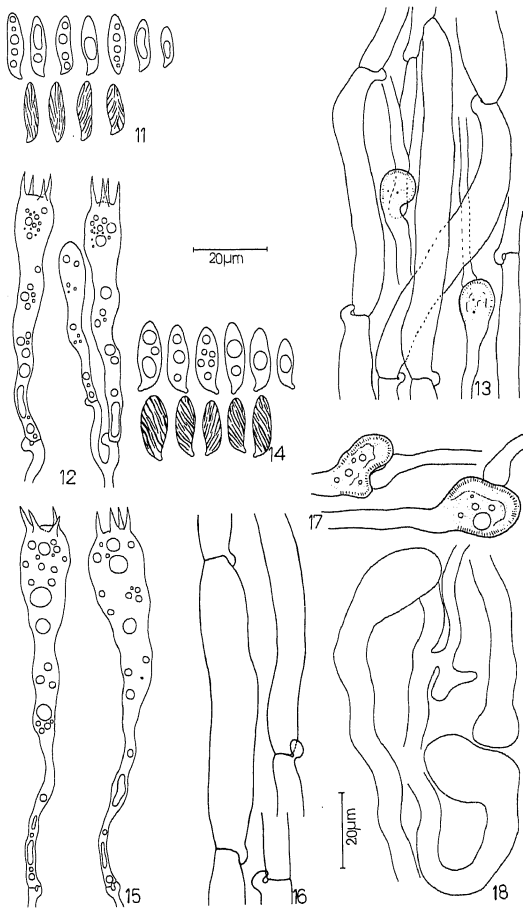
Hyphal system monomitic; hyphae up to $13.5 \mu\text{m}$ wide, clamped, wall slightly thickened; acyanophilous; ampullaeform swellings prominently ornamented; gloeoplerous hyphae common, up to $7 \mu\text{m}$ wide, inflated near the septa, thin-walled, cyanophilous. Basidia up to $95 \times 14 \mu\text{m}$, long clavate, multiguttulate, weakly cyanophilous, clamped, 4-spored; sterigmata up to $8.5 \mu\text{m}$ long. Basidiospores $14-16 (-17.5) \times 4.5-6.5 (-7.0) \mu\text{m}$, subcylindric to cylindric-ellipsoid, uni- to multiguttulate; wall moderately thickened, cyanophilous, spirally striated; apiculus up to $1.5 \mu\text{m}$ long.

Specimens examined: R. M. Sharda 22267 (PAN), on soil under coniferous forest, Chankaphug, Thimphu, Bhutan, September 23, 1980; R. M. Sharda 22278 (PAN), on soil under mixed forest, Nasephu, Thimphu, Bhutan, September 26, 1980.

Both the eastern Himalayan collections from Bhutan agree well with the concept of this species as given by Corner (1950) and Marr and Stuntz (1973). Characteristics that distinguish *R. strasseri* from the related species are pale yellow to light tan, massive fruit-bodies, bitter taste and prominently striated, large basidiospores.

2.5 *Ramaria clarobrunnea* Corner, Thind & Anand, Trans. Br. Mycol. Soc. 39: 478. 1956 (figures 19-24)

Fruit-bodies up to $16 \times 12 \text{ cm}$, massive, fleshy-watery, gregarious to scattered closely, orange white to pale orange, to light brown, unchanging on bruising; trunk up to $3 \times 3 \text{ cm}$, thick, smooth, white to pallid white; branches polychotomous below,



Figures 11–18. *R. botrytis*. 11. Basidiospores. 12. Basidia. 13. Context-hyphae and

Hyphal system monomitic; hyphae up to $12\text{ }\mu\text{m}$ wide, clamped, wall slightly thickened, acyanophilous; ampullaeform swellings conspicuously ornamented; gloeoplerous hyphae of two types: (i) short, unbranched, granular or soapy gloeoplerous hyphae marked by a main stem up to $6\text{ }\mu\text{m}$ wide from which are given out numerous small, narrow, usually simple branches; (ii) long, sparsely branched, non-granular or non-soapy gloeoplerous hyphae up to $8\text{ }\mu\text{m}$ wide, swollen near the septa, thin-walled, cyanophilous. Basidia $70\text{--}92 \times 10\text{--}13\text{ }\mu\text{m}$, long clavate, guttulate, clamped, 4-spored; sterigmata up to $7.5\text{ }\mu\text{m}$ long. Basidiospores $8.5\text{--}11.0\text{ (}\pm 12.0\text{)} \times 3.5\text{--}4.0\text{ (}\pm 5.0\text{)}\text{ }\mu\text{m}$, ellipsoid, uni- to multi-guttulate; wall thin to slightly thickened, cyanophilous, spirally striated; apiculus up to $1\text{ }\mu\text{m}$ long.

Specimens examined: R. M. Sharda 22113 (PAN), on soil under predominantly *Pinus kesiya* forest, Elephant falls, Shillong, Meghalaya, September 18, 1979; R. M. Sharda 22283 (PAN), on soil under mixed forest, Chailala, Paro, Bhutan, September 29, 1980.

This species was described by Corner *et al* (1956) on the basis of a single collection from Mussoorie (U.P.). Corner (1970) also included a collection from Panama under this species. During his various visits to different collecting sites in the north-western Himalayas, including the type locality, Khurana (1977) did not encounter this species there again.

In the eastern Himalayas, the species was collected twice from widely separated localities in shillong and Bhutan. Both these collections resemble well the holotype at PAN examined by Khurana (1977) and by us in having pale orange to light brown, massive fruit bodies, well developed gloeoplerous hyphal system (two types of gloeoplerous hyphae) and striated basidiospores.

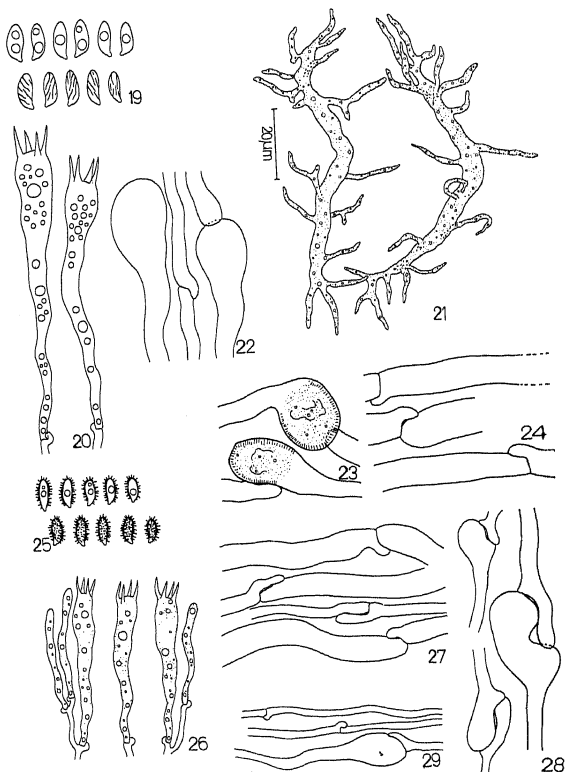
3. Subgenus—*Echinoramaria* Corner, Beih. Nova Hedwigia 33: 238–239. 1970.

3.1 *Ramaria flaccida* (Fr) Ricken, Vademecum. 254. 1918.

Fruit-bodies up to 8 cm long and 3.5 cm broad, medium to small sized, fleshy when fresh, drying brittle, solitary, gregarious; yellowish brown to brown; trunk indistinct to distinct, up to 2 cm long and 0.3 cm broad, slender; branches dichotomous, slender, profuse, lower branches up to 0.2 cm wide, smooth, becoming narrower and shorter upward, sometimes ultimate branches fused with one another, axils U-shaped; tips acute, minute, dichotomous or in unequal pairs, brown; flesh white to lighter concolorous; taste and smell not distinctive.

Hyphal system monomitic; hyphae up to $11\text{ (}\pm 12.5\text{)}\text{ }\mu\text{m}$ wide, clamped, thin-walled, acyanophilous; ampullaeform swellings abundant, smooth; hyphae of the rhizomorphs up to $3.5\text{ }\mu\text{m}$ wide, not inflated, clamped, thin-walled; ampullaeform swellings present, smooth. Basidia up to $50 \times 6.5\text{ }\mu\text{m}$, clavate, guttulate, clamped, 4-spored; sterigmata up to $4.5\text{ }\mu\text{m}$ long. Basidiospores $4.0\text{--}7.0 \times 3\text{--}3.5\text{ (}\pm 4.0\text{)}\text{ }\mu\text{m}$, ellipsoidal, uniguttulate; wall thin, cyanophilous, echinulate, spines up to $0.7\text{ }\mu\text{m}$ long; apiculus minute.

Specimens examined: R. M. Sharda 22286 (PAN), on humus and needle duff under



Figures 19–29. *R. clarobrunnea*. 19. Basidiospores. 20. Basidia. 21. Acanthodendroid gloeoplerous hyphae. 22. Normal gloeoplerous hyphae. 23. Ampullaeform swellings. 24. Context-hyphae. *R. flaccida* var. *longiramosa*. 25. Basidiospores. 26. Basidia. 27. Context-hyphae. 28. Smooth ampullaeform swellings. 29. Hyphae of the rhizomorphs.

In India, this species is first recorded by Thind and Anand (1956) from Mussoorie hills (U.P.). Subsequently, Thind (1961) and Khurana (1977) cited a number of collections from different localities in the north-western Himalayas indicating its common occurrence in that area.

As discussed by Corner (1950, 1970), and Thind (1961) it is quite a variable species, however, all the eastern Himalayan collections fall within the specific limits of this species. It is marked by small to medium size of the fruit-bodies (usually 4–8 cm long), greyish yellow to finally brown colour, non-bruising nature, and small, minutely echinulate basidiospores.

3.2 *Ramaria flaccida* var *longiramosa* Corner, Beih. Nova Hedwigia 33: 250, 1970 (figures 25–29)

Fruit-bodies up to 16 × 5 cm, fleshy-coriaceous, brittle after drying, gregarious or scattered closely, with abundant mycelial hyphae at the base; yellow to olive brown, unchanging on bruising; trunk up to 4 × 0.5 cm, slender, most part buried in the needle duff, white or light yellow; branches polychotomous below and dichotomous above, long, slender, smooth, axils broad; tips acute, dichotomous, light yellow; flesh dull white; taste and smell not distinctive.

Hyphal system monomitic; context-hyphae up to 10 µm wide, clamped, thin-walled, acyanophilous; ampullaeform swellings present, smooth; mycelial hyphae up to 3 µm wide, not inflated, clamped, thin-walled, ampullaeform swellings occur, smooth; gloeoplerous hyphae not observed. Basidia 35–45 × 5–6 µm, clavate, granular-guttulate, clamped, 4-spored; sterigmata up to 4.5 µm long. Basidiospores 5.5–7 (–8) × 3–4 µm, ellipsoid to pip-shaped, uniguttulate; thin to slightly thick-walled, cyanophilous, echinulate, spines dense, up to 1 µm long; apiculus up to 0.8 µm long.

Specimen examined: R. M. Sharda 22088 (PAN, SUCO), on needle duff under *Cryptomeria japonica* forest, Dhotrey, Darjeeling, West Bengal, September 10, 1979.

This taxon was described by Corner (1970) on the basis of a collection from Pangarango, Java. The fungus was not collected again after that and this is the second report to its occurrence in the world. Evidently, it is rare in its distribution.

The eastern Himalayan collection from Dhotrey (Darjeeling) fits well in the circumscription of the variety as given by Corner (1970). Microscopically, it cannot be separated from *R. flaccida* var *flaccida* but for the larger size of the fruit-bodies (up to 16 cm high).

3.3 *Ramaria sikkimia* Rattan & Khurana, Bibliotheca mycol. 66: 18. 1978.

Fruit-bodies up to 7 × 3 cm, small-sized, fleshy to fleshy-coriaceous, gregarious or scattered closely, brown, unchanging on bruising; trunk up to 2.5 × 0.6 cm, slender, pallid white; branches profuse, polychotomous below, dichotomous above, basal branches parallel, long, slender, becoming thinner and shorter upward, smooth, axils U-shaped; tips subacute to obtuse, paler concolorous to yellow; flesh light brown; taste and smell not distinctive.

Hyphal system monomitic; hyphae up to 8.5 µm wide, clamped, wall thin to slightly

hyphae fairly common, up to 3 μ m wide, simple or forked, swollen near the septa, thin-walled, cyanophilous. Basidia 40–52 \times 7–8.5 μ m, clavate, clamped, 3(2–4) spored; sterigmata up to 8 μ m long. Basidiospores up to 6.5–8.5 (–9) \times 4.5–5.5 (–6.2) μ m, broadly ellipsoid to subglobose, to lacrimiform, uniguttulate; wall up to 1.2 μ m thick, cyanophilous, prominently echinulate, spines up to 1.5 μ m long; apiculus up to 1.5 μ m long.

Specimens examined: R. M. Sharda 22072 (PAN, TENN), on soil under angiospermous forest, Takdah Cantt., Darjeeling, West Bengal, August 31, 1979; R. M. Sharda 22487 (PAN), on soil under broad-leaved forest, 3 km (Jamiri-Buragaon road), West Kameng, Arunachal Pradesh, September 13, 1981.

This species was described by Rattan and Khurana (1978) on the basis of a single collection from Manebhanjang, Darjeeling hills. During our search for the clavarioid fungi of the eastern Himalayas, we encountered this species in Darjeeling (West Bengal) and Jamiri (Arunachal Pradesh). Both these collections conform well with the holotype and its description as given by Rattan and Khurana (1978). However, the fruit-bodies are smaller (up to 7 \times 3 cm) than that of the holotype (up to 17 \times 5.5 cm) and appear to represent young fruit-bodies of this species. Therefore, more collections are needed to find out the exact range of variation of *R. sikkimia*. The underlining features of this species are the brown colour of the fruit-bodies with yellowish tips; unchanging flesh; predominantly 3-spored basidia, and broadly ellipsoid to subglobose to lacrimiform, prominently thick-walled, strongly echinulate basidiospores.

3.4 *Ramaria pusilla* (Peck) Corner, Ann. Bot. mem. 1: 617. 1950

Fruit-bodies up to 7 \times 3.5 cm, small-sized, fleshy-coriaceous, occurring singly, gregarious, arising from white mycelium, cream yellow to yellow ochraceous; colour changing to brownish on bruising; trunk up to 2 \times 0.5 cm, white to dull white; branching profuse, polychotomous below, dichotomous above, ascending, parallel, axils narrow or U-shaped; tips acute, minute, concolorous with the fruit-body surface; flesh white; taste and smell not distinctive.

Hyphal system monomitic; hyphae up to 10.5 μ m wide, clamped, wall thin, acyanophilous, ampullaeform swellings smooth; hyphae of the rhizomorphs up to 3 μ m wide, not inflated, clamped, thin-walled; ampullaeform swellings present, smooth; gloeoplerous hyphae not observed. Basidia up to 45 \times 6 μ m, clavate, guttulate, clamped, 4-spored; sterigmata up to 4.5 μ m long. Basidiospores 4.5–6 (–6.5) \times 2.5–3 (–3.5) μ m, ellipsoid or pip-shaped, uniguttulate, wall thin, cyanophilous echinulate, spines short, up to 0.5 μ m long, apiculus up to 0.5 μ m long.

Specimens examined: R. M. Sharda 22221 (PAN), on soil or bark-duff under predominantly *C. japonica* forest, Palmajuha, Darjeeling, West Bengal, August 30, 1980; R. M. Sharda 22255 (PAN), on leaf mold under broad-leaved forest, Uetselpong, Thimphu, Bhutan, September 21, 1980; R. M. Sharda 22333 (PAN), on bark-duff or soil under broad-leaved forest, Uetselpong, Thimphu, Bhutan, August 5, 1981; R. M. Sharda 22372 (PAN), on bark-duff under predominantly pine forest, Wagdiphodrang-Thimphu road forests, Bhutan, August 14, 1981.

R. pusilla was first recorded in India by Thind and Dev (1957) on the basis of two collections from Mussoorie (U.P.). Khurana (1977) added two more collections from

collections were found under coniferous forests. In collection number 22221, colour of the branches is yellow-ochraceous to honey yellow to oak brown, whereas collection numbers 22372 and 22333 are pale yellow to greyish yellow or creamish yellow in colour. Collection number 22221 develops dark-red coloration on bruising, whereas the other collections turn brownish on bruising. These variations, however, do not warrant distinction into separate taxa and all these collections have been treated under *R. pusilla*. Unifying characters which serve to recognise these collections as one taxon are the usually small fruit-bodies arising from basal mycelial mat; yellowish to dull yellowish colour of the branches; vivescent bruising reaction; and small, $4.5-6 \times 2.5-3$ (-3.5) μm , ellipsoid or pip-shaped, minutely echinulate basidiospores.

4. Subgenus: *Lentoramaria* Corner, Beih. Nova Hedwigia 33: 239. 1970

4.1 *Ramaria rubella* (Schaeffer per Krombholz) Petersen, Am. J. Bot. 61: 746. 1974.

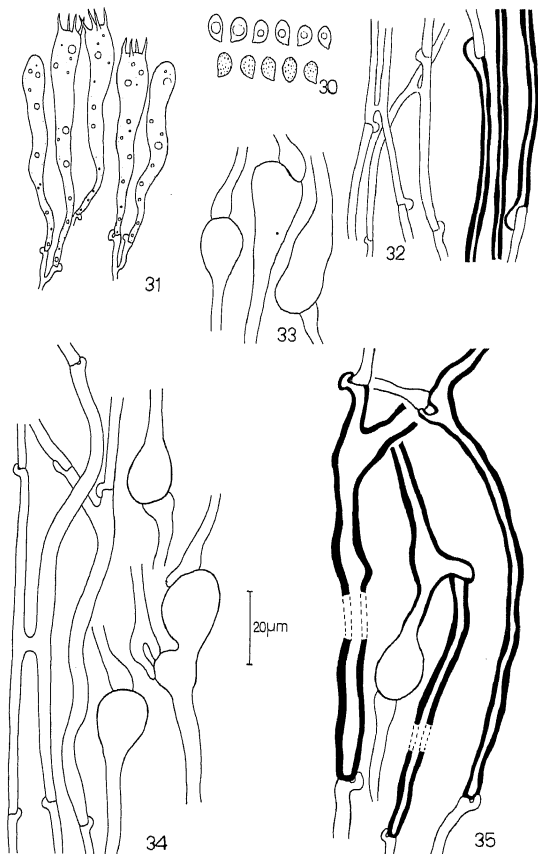
var *himalaica* Thind & Sharda, var. nov. (figures 30-35)

Fructus corpora ad 6×3.5 cm, lignicolus, ramificatus, caesnes ad carnem colorem et albi vertices. Truncus indistinctus ad distinctum. Systematicus hyphalus monomiticus cum intercalare cellae sclerificatae in contextu. Basidiospores $5-6.5$ (-7) $\times 3-4$ (-4.5) μm , exigua ellipotata ad ovoidatum, verruculosam.

Typus: In ligno tabo et acuus dufftus sub mixto silvae, Uetselpong, Thimphu, Bhutan, Aug. 5, 1981; Leg. R. M. Sharda 22332 (holotypus, PAN).

Fruit-bodies up to 6×3.5 cm, fleshy-tough, solitary gregarious, arising from white rhizomorphs; greyish orange or flesh coloured, unchanging on bruising; trunk indistinct to distinct, up to 1.5×0.4 cm, smooth, whitish; branching profuse, lax type, polychotomous below, dichotomous above, internodes in the basal branches up to 2 mm thick, long, slender, becoming thinner and shorter upward, axils U-shaped; tips acute, single or dichotomous, white; flesh whitish concolorous; taste and smell not distinctive.

Hyphal system monomitic both in rhizomorphs and fruit-body context; hyphae of the context of three types: (a) generative hyphae, up to $6 \mu\text{m}$ wide, clamped, thin-walled, acyanophilous; ampullaeform swellings present, smooth; (b) skeletalized generative hyphae, up to $7 \mu\text{m}$ wide, thick-walled, clamped, arising and ending in clamp connections, rarely ending blindly; and (c) intercalary sclerified cells, up to $8 \mu\text{m}$ wide, thick-walled, wall up to $1.2 \mu\text{m}$ thick, originating and ending with a clamp; hyphae of the rhizomorphs quickly turning pink when put in 10% KOH, two types of hyphae are present; (a) generative hyphae, up to $3 \mu\text{m}$ wide, not inflated, clamped, thin-walled, branched, ampullaeform swellings smooth; (b) sclerified generative hyphae, up to $3.5 \mu\text{m}$ wide, thick-walled, wall up to $0.8 \mu\text{m}$ thick, originating and ending in a clamp. Basidia up to $59 \times 8.5 \mu\text{m}$, clavate, guttulate, clamped, 4-spored; sterigmata up to $5 \mu\text{m}$ long. Basidiospores $5-6.5$ (-7) $\times 3-4$ (-4.5) μm , short ellipsoid to ovoid, uniguttulate; thin-walled, verruculose, warts minute, cyanophilous; apiculus up to $0.7 \mu\text{m}$ long.



Figures 30–35. *R. rubella* var. *himalaica*. 30. Basidiospores. 31. Basidia. 32. Hyphae of the rhizomorphs; generative hyphae and sclerified hyphae. 33. Smooth ampulliform hyphae. 34. Smooth ampulliform hyphae. 35. Sclerified hyphae.

Specimen examined: R. M. Sharda 22332 (PAN), on rotten wood and needle duff under mixed forest, Uetselpong, Thimphu Bhutan, August 5, 1981.

This variety is marked by having greyish orange to flesh coloured fruit-bodies, measuring up to 6×3.5 cm; rhizomorphic hyphae quickly turning pink in 10% KOH; monomitic hyphal system with sclerified cells in the context; and short ellipsoid to ovoid, minutely warted, $5-6.5 (-7) \times 3-4 (-4.5) \mu\text{m}$ basidiospores. In the similar change in colour of the rhizomorphic hyphae, in 10% KOH, it resembles *R. rubella* var *rubella* closely. However, the latter variety differs in its ruddy colour of the fruit-bodies, measuring up to 10 cm high; absence of sclerified cells in the context and slightly larger basidiospores measuring $6.3-9.5 \times 4.1-5.5 \mu\text{m}$ (fide Petersen 1975).

4.2 *Ramaria concolor* (Corner) Petersen, Bibliotheca mycol. 43: 54. 1975.

Fruit-bodies up to 8×6 cm, fleshy to fleshy tough, solitary, gregarious, arising from white, mycelial felt; flesh coloured to pinkish yellow above and ochraceous brown below, unchanging on bruising; trunk indistinct to distinct, up to 1×0.6 cm, smooth, white; branching profuse, polychotomous, internodes of lower branches up to 3 mm wide, stout, smooth, becoming narrower and shorter upward; axils U-shaped or broad; tips acute or subacute, single or in pairs, concolorous with the upper branches; flesh whitish concolorous; taste and smell not distinctive.

Hyphae of the context monomitic; three types of hyphae are present, (a) generative hyphae, up to $4 \mu\text{m}$ wide, clamped, thin-walled; (b) sclerified generative hyphae, up to $8.5 \mu\text{m}$ wide, clamped, wall up to $1.2 \mu\text{m}$ thick, composing major part of the context; (c) intercalary sclerified segments, up to $6.5 \mu\text{m}$ wide, thick-walled, wall up to $1 \mu\text{m}$ thick, arising and ending with a clamp; hyphae of the rhizomorphs dimitic; generative hyphae up to $3 \mu\text{m}$ wide, not inflated, clamped, thin-walled, branched, ampullaeform swellings present, smooth; skeletal hyphae up to $3 \mu\text{m}$ wide, long celled, aseptate, thick-walled, arising with a clamp and ending blindly. Basidia up to $53 \times 9 \mu\text{m}$, clavate, guttulate, clamped, 4-spored; sterigmata up to $5.5 \mu\text{m}$ long. Basidiospores $8.5 \times 4.2 \mu\text{m}$; $7.5-9 (-10) \times 3.5-4.5 \mu\text{m}$, ellipsoid to elongate ellipsoid, uni- to biguttulate; wall slightly thickened, obscurely ornamented, cyanophilous; apiculus up to $1 \mu\text{m}$ long.

Specimen examined: R. M. Sharda 22417 (PAN), on wood under broad-leaved forest, 10 km (Bomdila-Munna road), West Kameng, Arunachal Pradesh, August 29, 1981.

In India, this species was first recorded by Thind and Anand (1956) as '*R. stricta* var. *concolor*' on the basis of a collection from Mussoorie (U.P.). Petersen (1975) raised this to the rank of a species because it lacks yellow tips. *R. stricta* is marked by the possession of yellow tips. Khurana (1977) also referred to Mussoorie collection to *R. concolor*.

The only eastern Himalayan collection from Arunachal Pradesh fits well the circumscription of this species as given by Petersen (1975) and Khurana (1977). It is marked by flesh coloured to pinkish brown fruit-bodies with concolorous tips; lignicolous habit; dimitic rhizomorphic hyphae; and obscurely warted, ellipsoid basidiospores.

4.3 *Ramaria apiculata* (Fr.) Donk, Meded. bot. Mus. Herb. Rijks-Univ. Utrecht. 9: 105. 1933.

orange to brownish orange, or light violaceous brown at maturity, yellowish brown in the axils of lower branches, colour translucent on bruising; trunk indistinct or very small, up to 1 cm long and 5 mm broad, smooth, concolorous with the fruit-body surface; branching profuse, polychotomous, ascending, internodes long and up to 3 mm wide in the lower branches, shorter and thinner in the subsequent branches, axils U-shaped; tips minute, acute, in pairs or clustered, pale orange or concolorous with the branches; flesh paler concolorous or white; taste slightly bitter; smell not distinctive.

Hyphal system monomitic; context hyphae up to $10.5\text{ }\mu\text{m}$ wide, inflated, clamped, sparsely branched, thick-walled, wall up to $1.5\text{ }\mu\text{m}$ thick, acyanophilous; ampullaeform swellings present, prominently ornamented; hyphae of the rhizomorphs of two types; (a) generative hyphae, up to $3\text{ }\mu\text{m}$ wide, clamped, branched, thin-walled, ampullaeform swellings present, ornamented; (b) skeletalized generative hyphae up to $4\text{ }\mu\text{m}$ wide, clamped, thick-walled, wall up to $0.8\text{ }\mu\text{m}$ thick. Basidia up to $60 \times 9\text{ }\mu\text{m}$, clavate, guttulate, clamped, 4-spored; sterigmata up to $6.5\text{ }\mu\text{m}$ long. Basidiospores $8.5 \times 4\text{ }\mu\text{m}$; $7-9(-9.5) \times 3-4(-4.5)\text{ }\mu\text{m}$, ellipsoid, uni- to biguttulate; thin to slightly thick-walled, verruculose, warts prominent, cyanophilous; apiculus up to $1\text{ }\mu\text{m}$ long.

Specimens examined: R. M. Sharda 22331 (PAN), on wood under broad-leaved forest, Uetselpong, Thimphu, Bhutan, August 5, 1981; R. M. Sharda 22353 (PAN), on decayed wood under mixed forest, D'Dzong, Paro, Bhutan, August 9, 1981.

This species was first recorded by Thind (1961) on the basis of four collections from Simla hills (H.P.). Subsequent collections made by other workers and Khurana (1977) from different localities in the north-western Himalayas indicate its common occurrence under the *Cedrus deodara* forests of this region.

Both the eastern Himalayan collections made under the angiospermous or mixed forests in Bhutan, agree well their north-western Himalayan counterparts. The species is marked by slender fruit-bodies with ascending branches; greyish orange, brownish orange or light violaceous brown fruit-bodies with paler concolorous tips; monomitic hyphal construction with prominently thick-walled hyphae; and verruculose basidiospores.

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References

- Corner E J H 1950 A monograph of *Clavaria* and allied genera *Ann. Bot. Mem.* (London: Oxford University Press) vol. 1, pp. 740
Corner E J H 1970 Supplement to *A monograph of Clavaria and allied genera.*, *Beih. Nova Hedwigia* 33 1-299

- Corner E J H, Thind K S and Anand G P S 1956 The Clavariaceae of the Mussoorie Hills (India). II; *Trans. Br. Mycol. Soc.* **39** 475-484
- Holmgren P K and Keuken W 1974 *Index Herbariorum, Part I. The herbaria of the world*, 6th ed. (Regnum Veg.) **92** 397 pp.
- Khurana I P S 1977 *Studies on the clavarioid fungi of India*. Ph.D. thesis, Panjab University, Chandigarh, India
- Kornerup A and Wanscher J H 1967 *Methuen handbook of colour*. (London: Methuen & Co.) pp. 243
- Marr C D and Stuntz D E 1973 *Ramaria* of western Washington; *Bibliotheca Mycol.* **38** 232
- Petersen R H 1975 *Ramaria* subgenus *Lentoramaria* with emphasis on North American taxa; *Bibliotheca Mycol.* **43** 1-161
- Rattan S S and Khurana I P S 1978 The clavarias of the Sikkim Himalayas; *Bibliotheca Mycol.* **66** 1-68 pp
- Thind K S 1961 *The Clavariaceae of India* (New Delhi: Indian Council Agri. Res.) pp. 197
- Thind K S and Anand G P S 1956 The Clavariaceae of the Mussoorie Hills I; *J. Indian Bot. Soc.* **35** 92-102
- Thind K S and Dev S 1957 The Clavariaceae of the Mussoorie Hills. VIII; *J. Indian Bot. Soc.* **36** 475-485

Induction of hyphal branching in *Bipolaris sorokiniana* by sodium chloride

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Abstract. The hyphae of *Bipolaris sorokiniana* (Sacc. in Sorok) Shoemaker, a fungus belonging to the hyphomycetes, branched profusely while growing on a medium containing sodium chloride. Non-ionic osmotic compounds and other salts failed to induce branching. It appears that the Na ion of NaCl is responsible for inducing the hyphae to branch.

Keywords. Hyphal branching; *Bipolaris sorokiniana*; sodium chloride.

1. Introduction

Fungi are known to branch profusely when exposed to osmotic shock (Katz and Rosenberger 1971; Trinci and Collinge 1974). Osmotic shock is usually provided by growing the fungus in a high-salt medium and then suspending the growth in distilled water. We found that when *Bipolaris sorokiniana* (Sacc. in Sorok) Shoemaker was grown on a high-salt medium as a pretreatment to subjecting the fungus to osmotic shock, it branched profusely. This paper presents the details of salt-induced branching in *B. sorokiniana*.

2. Materials and methods

A single-spore isolate of the fungus was used. A plug of mycelium cut from the growing margin of 3-day old colony on Czapek-Dox agar medium was used as inoculum. This plug was placed on a disk of cellophane overlying Czapek agar medium or on Czapek agar medium containing NaCl, KCl, Na₂SO₄, BaCl₂, MgCl₂, CaCl₂, CaCO₃, or CH₃COONa in various concentrations. The pH of each medium was adjusted to 6.5 before autoclaving. The number of hyphal tips formed was counted after 48 hr of incubation at $30 \pm 1^\circ\text{C}$ by observing the colony margin under the low power field of a compound microscope. Osmotic shock was provided by growing the fungus on cellophane overlaying NaCl medium for 48 hr and then by suspending the growth in distilled water for 10 min. The growth was then transferred to normal Czapek agar medium and observed after incubating for 2 hr. Statistical analyses were carried out using the Student's *t* test.

3. Results and discussion

The effects of 0.17, 0.34, 0.68 and 1.02 M NaCl on the branching of the fungus were studied. The rate of hyphal elongation and therefore the growth was progressively

medium showed extensive subapical branching. The number of branches produced by the hyphae was significantly increased by NaCl at and above 0.34 M concentration (table 2)

To test if the branching induced by NaCl is due to an osmotic phenomenon, the effect of some non-ionic substances was studied. The fungus was grown for 48 hr on Czapek agar medium containing glycerol or mannitol at concentrations iso-osmotic with 0.68 M NaCl and the branching assessed. The non-ionic compounds failed to increase the branching potentiality of the fungus (table 3).

Since NaCl induced profuse branching of hyphae, the effect of KCl, Na₂SO₄, MgCl₂, CaCO₃, CaCl₂ and CH₃COONa on hyphal differentiation was studied. The fungus was grown for 48 hr on Czapek agar medium containing different concentrations of these salts. The growth of the fungus progressively decreased with increasing concentrations of the salts (figure 1). The growth was totally inhibited by 0.34 M BaCl₂. NaCl induced maximum branching at 0.68 M concentration. Branching was significantly increased by Na₂SO₄ at 0.34 and 0.68 M and CH₃COONa at 0.68 M. The other salts failed to increase the number of branches (figure 1). (CaCO₃ at 1.02 M induced some branching!).

The reduced growth of the fungus on a high-salt medium (figure 1) could be due to

Table 1. Influence of osmotic shock on the growth of *B. sorokiniana*

Concentration of NaCl (M)	Diameter of the colony (mm)		Rate of hyphal elongation ($\mu\text{m min}^{-1}$)	
	Before shock	After shock	Before shock	After shock
0	22.5	25.5	4.0	5.6
0.17	18.0	22.0	2.4	4.0
0.34	11.0	14.0	1.6	4.0
0.68	9.5	13.0	0.8	3.6
1.02	7.0	10.5	1.2	4.0

Table 2. Effect of different concentrations of NaCl on the branching of hyphae of *B. sorokiniana*

Concentration of NaCl (M)	No. of hyphal tips*
0	630.6
0.17	858.9
0.34	2930.7
0.68	3669.2
1.02	2235.8
Critical difference at 5% level	1579.8

* Per unit width (1 cm) of margin of colony after 48 hr growth

Compounds	No. of hyphal tips*
Control	706.1
Mannitol	464.1
Glycerol	617.1
NaCl	3621.6
Critical difference at 5% level	248.7

* Per unit width (1 cm) of margin of colony after 48 hr growth.

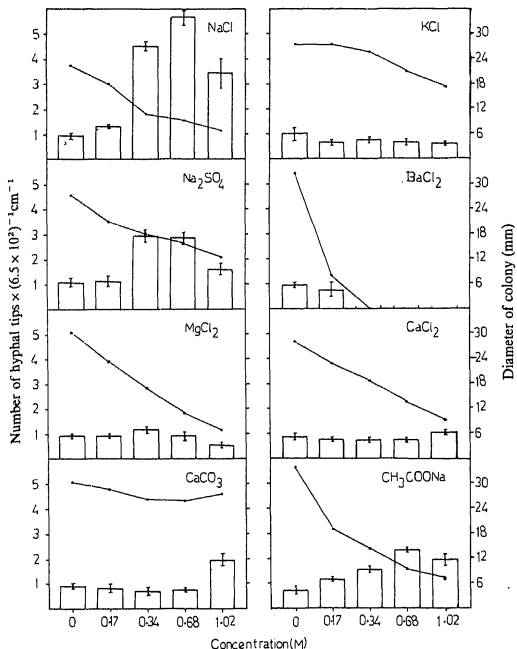


Figure 1. Effect of salts on the growth and branching of hyphae of *Bipolaris sorokiniana*. Bars show number of branches (with standard deviation) and curve shows growth.

restored (table 1). Turgor plays an important role in hyphal elongation (Robertson 1968). Hyphal growth is known to stop with the application of hypertonic solutions and to resume when turgor is restored (Park and Robinson 1966b). However, the increased branching induced by NaCl (table 2) is not an osmotic phenomenon as non-ionic osmotic compounds did not increase the number of branches (table 3).

It is evident that the branching is induced by Na^+ of NaCl and not by Cl^- since other chloride salts failed to increase the branching potentiality of the fungus. The increased branching in the presence of Na_2SO_4 and CH_3COONa also indicates this phenomenon (figure 1).

Developing systems drive steady ion currents through themselves thereby providing substantial electrical field within themselves (Jaffe and Nuccitelli 1977). It has been shown that mycelial fungi also generate steady electrical currents around their hyphal tips (Gow 1984; Horwitz *et al* 1984) and these currents may be responsible for transporting the vesicles containing cell wall materials to the hyphal tip. Gooday (1983) opined that a bioelectrical current acting either electrophoretically on such vesicles or through an electro-osmotic flow could be the driving force in vesicle transport of hyphae. He also suggested that there is an ionic current entering the tip of the hypha and leaving it in subapical regions. This has been proved using a vibrating probe (Gow 1984). The presence of Na^+ in the external medium could specifically interfere with such currents thereby retarding or stopping vesicle flow in the hypha. This could lead to profuse branching of hyphae since an event which stops or reduces transport of vesicles is likely to result in branch formation (Trinci 1979).

It is relevant to mention here the work of Jennings (1979). He showed that the hyphal tip of *Dendryphiella salina* was much less selective for potassium against sodium than those regions behind the tip and that the ATPase activity in the fungus was enhanced by Na^+ .

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References

- Gooday G W 1983 The hyphal tip; In *Fungal differentiation—A contemporary synthesis* (ed) J E Smith (New York: Marcel Dekker) pp. 315–356
- Gow N A R 1984 Transhyphal electrical currents in fungi; *J. Gen. Microbiol.* **130** 3313–3318
- Horwitz B A, Weisenseel M M, Dorn A and Gressel J 1984 Electric currents around growing *Trichoderma* hyphae before and after autoinduction of conidiation; *Plant Physiol.* **74** 912–916
- Jaffe L F and Nuccitelli R 1977 Electrical controls of development; *Annu. Rev. Biophys. Bioeng.* **6** 445–476
- Jennings D H 1979 Membrane transport and hyphal growth; In *Fungal walls and hyphal growth* (eds) J H Burnett and A P J Trinci (London: Cambridge University Press) pp. 279–294
- Katz D and Rosenberger R F 1971 Hyphal wall synthesis in *Aspergillus nidulans*. Effect of protein synthesis inhibition and osmotic shock on chitin insertion and morphogenesis; *J. Bacteriol.* **108** 184–190
- Park D and Robinson P M 1966b Aspects of hyphal morphogenesis in fungi; In *Trends in plant morphogenesis* (ed) E G Cutter (London: Longman Green) pp. 27–44

- Robertson N F 1968 The growth process in fungi; *Annu. Rev. Phytopathol.* **6** 115–136
- Trinci A P J 1979 The duplication cycle and branching in fungi; In *Fungal walls and hyphal growth* (eds) J H Burnett and A P J Trinci (London; Cambridge University Press) pp. 319–358
- Trinci A P J and Collinge A J 1974 Occlusion of septal pores of damaged hyphae of *Neurospora crassa* by hexagonal crystals; *Protoplasma* **80** 56–67

Assimilate supply in relation to grain weight in wheat (*Triticum aestivum* L)

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Abstract. The contents of various free sugars in large and small wheat grains were compared with those in normal grains at different stages of grain development. Their content paralleled grain weight only upto 14 days after anthesis (A + 14). On a dry weight basis, no positive correlations were observed between the concentrations of various free sugars and grain weight at any of the stages. Differences in grain weight were dependent mainly on the number of cells formed in the endosperm which in turn seemed to be regulated by the assimilate supply available to the grain during the first 14 DAA.

Keywords. *Triticum aestivum* L; free sugars; sucrose; starch; grain weight.

1. Introduction

Grain weight is an important component of yield in cereals including wheat (*Triticum aestivum* L), but the physiological and biochemical factors limiting grain size are still matters of conjecture. It is believed that either the source *via* the supply of precursors, or the sink by virtue of its capacity to accept assimilates and further converting them to starch are the main factors limiting starch accumulation and hence grain weight. However, the relative importance of source and sink in controlling grain weight in relation to yield is still controversial (Thorne 1974; Gieger 1976). Defoliation and shading (Asana *et al* 1969; Ford and Thorne 1975; Sofield *et al* 1977), altering grain number per ear (Bingham 1967; Bremner and Rawson 1978; Radley 1978) or a combination of both (Pinthus and Millet 1978; Jenner 1979, 1980) are the common techniques employed in studies designed to differentiate between the two. The primary assumption implied in defoliation is that it limits grain growth by reducing the supply of assimilates. Degraining, on the other hand, is assumed to cause large grains by increasing the supply of assimilates to the remaining grains. Because of the scarcity of information on the effects of these treatments on the levels of soluble carbohydrates in the grain, the validity of these assumptions could not be ascertained unequivocally. An attempt has, therefore, been made here to quantify various sugars in larger and smaller grains resulting from degraining and defoliation, and to compare the results with those obtained from normal grains in order to test the validity of the assumption that assimilate supply may limit starch accumulation and so control grain weight.

2. Materials and methods

Wheat plants (cv WH-157) were raised under field conditions, following recommended agronomic practices, in three replications (Kumar and Singh 1980, 1981). In all, there

represented in three. At anthesis, ears from 150 plants from each replication were trimmed to eight central spikelets (degraining); the flag and penultimate leaves were removed from another 150 plants (defoliation). From each replication, duplicate samples were taken and individually analysed. Hence, each value included in the tables is a mean of six independent estimations.

The grain samples, harvested at weekly intervals from A + 7 until A + 42 were dried in an oven at 105 °C for 4 hr and then at 80 °C to constant dry weight. Only the central grains from each spikelet were used for further analysis. Soluble sugars were extracted by boiling the powdered sample in 80 % (v/v) ethanol (Cerning and Guilbot 1973). The method of Yemm and Willis (1954) was employed for the determination of total soluble sugars. Reducing sugars were estimated by Somogyi's modified method (Nelson 1944; Somogyi 1945). The content of nonreducing sugars was obtained as the difference between total and reducing sugars. Sucrose was estimated as reducing sugars after hydrolysis with invertase (Johnson *et al* 1964). Starch from the sugar-free pellet was estimated by the method of Clegg (1956). The number of endosperm cells was counted following the method of Rijven and Wardlaw (1966). Statistical analysis was performed by the methods described by Snedecor (1956).

3. Results and discussion

For the sake of convenience, heavier and lighter grains resulting from degraining and defoliation have respectively been referred to as H and L.

Dry weight increased continuously throughout grain development (table 1). It was greatest in H and least in L with control grains having intermediate values, indicating

Table 1. Dry weight of developing wheat grains of variety WH-157 under defoliation (L) degraining (H) and normal development (C)^a

Days after anthesis	Dry weight (mg/grain)		
	L	C	H
7	2.7 ±0.08	3.3 ±0.08	4.3 ±0.08
14	9.7 ±0.35	11.0 ±0.35	12.2 ±0.40
21	18.9 ±0.32	20.6 ±0.36	25.4 ±0.34
28	26.3 ±0.69	33.0 ±0.69	40.2 ±0.82
35	42.0 ±0.84	46.3 ±0.78	52.9 ±0.71
42	42.8 ±1.12	50.1 ±1.07	60.1 ±1.24

^a values are mean ± S.E. from six independent estimations.

($P < 0.01$) altered by defoliation and degreening. At maturity, H grains had 20 % more and L 14 % less dry matter than the control.

Starch content grain⁻¹ increased progressively throughout development (table 2); rapid starch synthesis commencing at 14 days after anthesis. Its amount was lowest in L and highest in H at all stages of development. The rate of starch accumulation during the linear phase of grain filling (days 14 to 35) for the three treatments, L, control and H respectively was 1.39, 1.55 and 1.79 mg grain⁻¹ day⁻¹. However, in the last interval (days 35 to 42), the mean daily rate of starch accumulation was 0.1, 0.46 and 0.88 mg day⁻¹ in three types, indicating that the treatments appeared to have affected the duration of grain filling. The responses also indicated the existence of a mechanism which could influence the capacity of endosperm to accumulate starch. Responses to degreening showed that grains had the capacity to grow longer than normal in intact ears, exhibiting the presence of unused growth potential. Bremner and Rawson (1978) also proposed the concept of unused growth potential in developing cereal grains.

Sucrose content grain⁻¹ increased sharply at day 14 after anthesis, declining thereafter upto day 28 and then remaining almost constant till the end (table 3). Except at the 35 day stage, its content grain⁻¹ was higher in H than in control. Control grains likewise had a higher sucrose content grain⁻¹ than L apart from the 21-day stage. However, a significant positive correlation ($P < 0.01$) between sucrose content and grain weight was observed only at 14 DAA. Sucrose per unit dry weight decreased progressively with maturity and at no stage after day 14 was there a significant positive correlation between sucrose concentration and grain weight.

Total soluble sugars content grain⁻¹ was significantly higher in H than in control and L grains until the 14-day stage (table 4). After 14 days also, although the pattern was similar the differences were non-significant. Reducing sugars grain⁻¹ followed a developmental pattern similar to that obtained for sucrose and total soluble sugars

Table 2. Starch content in developing wheat grains of variety WH-157 under defoliation (L) degreening (H) and normal development (C)^a

Days after anthesis	mg/grain			mg/g dry weight		
	L	C	H	L	C	H
7	0.47 ±0.01	0.60 ±0.02	0.83 ±0.02	172.1 ±3.67	182.2 ±5.06	191.3 ±4.56
14	3.11 ±0.09	3.64 ±0.11	4.12 ±0.12	321.6 ±9.55	332.5 ±10.26	337.1 ±9.91
21	12.10 ±0.17	13.40 ±0.20	17.00 ±0.47	641.3 ±9.05	652.0 ±10.06	669.8 ±18.56
28	19.66 ±0.27	24.80 ±0.32	30.37 ±0.63	748.1 ±10.66	751.6 ±9.56	754.9 ±15.81
35	32.34 ±0.80	36.17 ±0.74	41.66 ±0.81	770.1 ±19.05	781.3 ±15.95	788.1 ±15.30
42	32.97 ±0.47	39.36 ±0.78	47.86 ±1.24	770.3 ±10.90	785.1 ±15.61	797.2 ±20.61

^a values are mean ± S.E. from six independent estimations.

under defoliation (L), degreening (H) and normal development (C)

Days after anthesis	mg/grain			mg/g dry weight		
	L	C	H	L	C	H
7	0.60 ±0.01	0.76 ±0.02	1.04 ±0.01	219.6 ±2.10	232.6 ±4.15	241.6 ±2.31
14	1.25 ±0.01	1.37 ±0.04	1.50 ±0.03	129.2 ±1.12	125.4 ±3.07	122.8 ±2.82
21	1.03 ±0.03	0.96 ±0.07	1.24 ±0.06	54.5 ±1.53	46.5 ±3.10	48.9 ±2.35
28	0.58 ±0.03	0.75 ±0.03	0.83 ±0.03	22.2 ±1.15	22.8 ±0.87	20.6 ±0.90
35	0.69 ±0.05	0.76 ±0.05	0.62 ±0.05	16.5 ±1.06	16.5 ±0.95	11.8 ±0.97
42	0.68 ±0.07	0.80 ±0.08	0.94 ±0.09	15.9 ±1.61	15.9 ±1.96	15.7 ±1.66

^a values are mean ± S.E. from six independent estimations.

Table 4. Total soluble sugars content in developing wheat grains of variety WH-157 under defoliation (L) degreening (H) and normal development (C)^a

Days after anthesis	mg/grain			mg/g dry weight		
	L	C	H	L	C	H
7	1.27 ±0.02	1.59 ±0.05	2.22 ±0.06	461.6 ±11.85	484.0 ±15.75	514.0 ±14.7
14	2.80 ±0.09	3.68 ±0.12	4.23 ±0.12	288.8 ±9.15	336.1 ±10.80	346.3 ±9.25
21	2.44 ±0.09	2.45 ±0.14	3.14 ±0.17	129.1 ±4.55	118.9 ±6.85	123.6 ±6.95
28	2.01 ±0.12	2.23 ±0.15	2.45 ±0.15	76.4 ±4.56	67.7 ±4.45	60.8 ±3.70
35	1.89 ±0.12	2.19 ±0.21	2.35 ±0.21	45.0 ±3.00	47.4 ±4.55	44.4 ±4.11
42	1.47 ±0.10	1.54 ±0.13	1.81 ±0.11	34.3 ±2.40	30.7 ±2.55	30.2 ±1.82

^a values are mean ± S.E. from six independent estimations.

(table 5). Though at most of the stages, H grains had the greatest control intermediate and L the least quantities of non-reducing sugars grain⁻¹ (table 6), the differences again were significant ($P < 0.01$) only upto the 14-day stage. On a dry weight basis, parallelism between non-reducing sugars and grain size was obtained only on day 7. However, the differences between the treatments were non-significant.

The results reported above indicate that though the levels of total, reducing and non-reducing sugars grain⁻¹ were altered as a result of defoliation or degreening, the

(L), degreening (H) and normal development (C)^a

Days after anthesis	mg/grain			mg/g dry weight		
	L	C	H	L	C	H
7	0.22 ± 0.01	0.28 ± 0.00	0.43 ± 0.01	79.3 ± 3.61	87.3 ± 0.60	100.5 ± 1.40
14	0.52 ± 0.04	0.71 ± 0.04	0.94 ± 0.01	53.3 ± 3.85	65.0 ± 3.35	77.2 ± 0.31
21	0.53 ± 0.02	0.58 ± 0.02	0.70 ± 0.02	28.3 ± 0.80	28.3 ± 0.95	27.8 ± 0.82
28	0.41 ± 0.01	0.51 ± 0.01	0.65 ± 0.02	15.8 ± 0.17	15.4 ± 0.36	16.2 ± 0.40
35	0.37 ± 0.01	0.40 ± 0.01	0.50 ± 0.02	8.8 ± 0.26	8.6 ± 0.21	9.5 ± 0.36
42	0.35 ± 0.01	0.39 ± 0.02	0.46 ± 0.02	8.1 ± 0.21	7.7 ± 0.36	7.7 ± 0.32

^a values are mean ± S.E. from six independent estimations.**Table 6.** Nonreducing sugars content in developing wheat grains of variety WH-157, under defoliation (L), degreening (H) and normal development (C)^a

Days after anthesis	mg/grain			mg/g dry weight		
	L	C	H	L	C	H
7	1.05 ± 0.03	1.30 ± 0.03	1.79 ± 0.03	328.3 ± 10.70	396.7 ± 9.12	413.5 ± 6.66
14	2.29 ± 0.08	2.97 ± 0.05	3.25 ± 0.06	236.5 ± 9.00	271.1 ± 5.02	269.1 ± 4.60
21	1.90 ± 0.06	1.86 ± 0.04	2.43 ± 0.08	100.8 ± 3.30	90.6 ± 1.90	85.8 ± 3.30
28	1.59 ± 0.07	1.73 ± 0.10	1.80 ± 0.12	60.6 ± 2.46	52.3 ± 3.05	44.7 ± 2.90
35	1.52 ± 0.10	1.79 ± 0.11	1.84 ± 0.15	36.2 ± 2.42	38.8 ± 2.40	34.9 ± 2.80
42	1.12 ± 0.08	1.15 ± 0.05	1.34 ± 0.09	26.2 ± 1.90	23.0 ± 1.16	22.4 ± 1.50

^a values are mean ± S.E. from six independent estimations.

differences between treatments were significant only upto the 14-day stage. Sucrose is the principal form in which carbohydrates are translocated to the grain via the phloem (Porter 1962). Its level, therefore, is a more reliable and stable indicator for predicting altered assimilate supply. The pattern of sucrose content in different grain types was similar to that shown by other sugars, indicating that defoliation cuts down the supply of assimilates available for distribution to grains and that the reduction in the rate of

supply of assimilates. The responses to removing spikelets are also a pointer in the same direction.

Since the % water content of the three grain types did not differ much throughout development, expression of the results on a dry weight basis was considered to reflect the behaviour of apparent concentrations of sugars quite accurately. On dry weight basis, at none of the stages, a significant positive correlation was obtained between grain weight and any of the sugars. Jenner (1979) also found apparent concentrations of sucrose in the endosperm of wheat to remain unaffected by degreining. Such a relationship invalidates the assumption that changes in the rate of starch accumulation and final grain size are attributable only to the altered inflow of assimilates into the grain. It can thus be concluded that the accumulation of starch in developing wheat grains is not limited at the level of supply of precursors. Additional support comes from the data on a per grain basis which show that significant alterations in the levels of various free sugars were observed only up to the 14-day stage, whereas rapid starch synthesis commenced from 14 days onwards.

Endosperm cell number in wheat is known to be fixed by $A + 14$ and the further increase in grain size is by cell expansion (Jennings and Morton 1963). Estimates of endosperm cell numbers were therefore made at the 28-day stage. Table 7 shows a significant positive correlation ($P < 0.01$) between endosperm cell number and final grain dry weight. However, it is obvious from the cell weight data (table 7) that variation in grain weight is not accounted for entirely by variation in endosperm cell number.

Deposition of dry matter in the endosperm of cereal seeds is generally viewed as the product of the number of cells in the endosperm and weight per cell. Of these two components, cell division dominates the first half of the period of grain growth while growth in the second half results from the deposition of dry matter in cells produced in the first half. It is therefore not surprising that a positive relationship has been obtained here between final grain dry weight and endosperm cell number. A similar relationship has been found to be true in varietal (Brocklehurst 1977) and positional differences of grain (Singh and Jenner 1982) and in factors such as degreining (Brocklehurst 1977; Radley 1978) and shading (Wardlaw 1970), supporting the idea put forward earlier by Bingham (1969) that the capacity of the endosperm to accumulate dry matter is determined by the number of cells formed in the endosperm. From a detailed study of culturing the detached wheat ears in solutions of sucrose and glutamine or exposing the intact plants to high or low photon irradiance, Singh and Jenner (1984) also concluded that the rate of cell division in the endosperm and thus the capacity of the endosperm to

Table 7. Endosperm cell number and average cell weight in wheat grains of variety WH-157 on day 28^a

Grain type	Endosperm cell number	Average cell weight (mg cell ⁻¹)
L (defoliated plants)	133600 ± 2660	197 × 10 ⁻⁶
C (normal plants)	146200 ± 3220	226 × 10 ⁻⁶
H (degreined plants)	162400 ± 2740	246 × 10 ⁻⁶

^a values are mean ± S.E. of six independent estimations.

supply of organic nutrients from the rest of the plant. However, they could not decipher the nature of the mechanism controlling the above process.

Putting all the information together, it could be proposed that the cell number in the wheat endosperm is a major factor controlling final grain dry weights. This number seems to be regulated through the supply of assimilates to the grain during the cell division phase which continues in wheat upto 14 days after anthesis. The endosperm cell number changes to match the amount of available assimilates and in turn determine the final grain weight.

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References

- Asana R D, Parvatikar S R and Saxena N P 1969 Studies on physiological analysis of yield IX. Effect of light intensity on the development of the wheat grain; *Physiol. Plant.* **22** 915–924
- Bingham J 1967 Investigations on the physiology of yield in winter wheat by comparison of varieties and by artificial variation in grain number per ear; *J. Agric. Sci.* **68** 411–422
- Bingham J 1969 The physiological determinants of grain yield in cereals; *Agric. Prog.* **44** 30–42
- Bremner P M and Rawson H M 1978 The weights of individual grains on the wheat ear in relation to their growth potential, the supply of assimilate and interaction between grains; *Aust. J. Pl. Physiol.* **5** 61–72
- Brocklehurst P A 1977 Factors controlling grain weight in wheat; *Nature (London)* **266** 348–349
- Cerning J and Guilbot J 1973 Changes in carbohydrate components during maturation in wheat and barley kernel; *Cereal Chem.* **50** 220–232
- Clegg K M 1956 The application of the anthrone reagent for the estimation of starch in cereals; *J. Sci. Food Agric.* **7** 40–44
- Ford M A and Thorne G N 1975 Effects of variation in temperature and light intensity at different times on growth and yield of spring wheat; *Ann. Appl. Biol.* **80** 283–299
- Gieger D R 1976 Effects of translocation and assimilate demand on photosynthesis; *Can. J. Bot.* **54** 2337–2345
- Jenner C F 1979 Grain filling in wheat plants shaded for brief periods after anthesis; *Aust. J. Pl. Physiol.* **6** 629–641
- Jenner C F 1980 Effects of shading or removing spikelets in wheat: testing assumptions; *Aust. J. Pl. Physiol.* **7** 113–121
- Jennings A C and Morton R K 1963 Changes in carbohydrate, protein and non protein nitrogenous compounds of developing wheat grain; *Aust. J. Biol. Sci.* **16** 318–331
- Johnson G, Lambert G, Johnson D K and Sunderwirth S C 1964 Plant tissue analysis. Colorimetric determination of glucose, fructose and sucrose in plant material using a combination of enzymatic and chemical methods; *J. Agric. Food Chem.* **12** 216–219
- Kumar R and Singh R 1980 The relationship of starch metabolism to grain size in wheat; *Phytochemistry* **19** 2299–2303
- Kumar R and Singh R 1981 Free sugars and their relationship with grain size and starch content in developing wheat grains; *J. Sci. Food Agric.* **32** 229–234
- Nelson N 1944 A photometric adaptation of the Somogyi method for the determination of glucose; *J. Biol. Chem.* **153** 375–380
- Pinthus M J and Millet E 1978 Interactions among number of spikelets, number of grains and grain weight in the spikes of wheat (*Triticum aestivum* L.); *Ann. Bot.* **42** 839–848
- Porter H K 1962 Synthesis of polysaccharides of higher plants; *Annu. Rev. Pl. Physiol.* **13** 303–328
- Radley M 1978 Factors affecting grain enlargement in wheat; *J. Exp. Bot.* **29** 919–934

- Rijven A H G C and Wardlaw I F 1966 A method for the determination of cell number in plant tissues; *Exp. Cell Res.* **41** 324–328
- Singh B K and Jenner C F 1982 Association between concentrations of organic nutrients in the grain, endosperm cell number and grain dry weight within the ear of wheat; *Aust. J. Pl. Physiol.* **9** 83–95
- Singh B K and Jenner C F 1984 Factors controlling cell number and grain dry weight in wheat: Effects of shading on intact plants and of variation in nutritional supply to detached, cultured ears; *Aust. J. Pl. Physiol.* **11** 151–163
- Snedecor G W 1956 *Statistical methods* (Ames: Iowa State University) 5th ed.
- Sofield I, Evans L T, Cook M G and Wardlaw I F 1977 Factors affecting the rate and duration of grain filling in wheat; *Aust. J. Pl. Physiol.* **4** 785–797
- Somogyi M 1945 A new reagent for the determination of sugars; *J. Biol. Chem.* **160** 61–69
- Thorne G N 1974 Physiology of grain yield of wheat and barley. Report of Rothamsted Experimental Station for 1973, part 2, pp 5–25
- Wardlaw I F 1970 The early stages of grain development in wheat: response to light and temperature in a single variety; *Aust. J. Biol. Sci.* **23** 765–774
- Yemm E W and Willis A J 1954 The estimation of carbohydrates in plant extracts by anthrone; *Biochem. J.* **57** 508–514

Effect of established populations of two annual weeds on their newly emerged seedling cohorts

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Abstract. The effect of established populations of two sympatric annual weeds, *Galinsoga ciliata* (Raf) Blake and *G. parviflora* Cav on their newly emerged seedling cohorts was studied. These weeds occur abundantly in croplands and early successional communities in the north-eastern hill region of India. Seed germination, survivorship and growth in both cases were reduced owing to competition from their established plants. The established cohorts of *G. ciliata* exercised greater suppressive effect than those of *G. parviflora*. Twenty five-day old cohort of *G. ciliata* caused maximum suppression on foliage production, seed output and dry matter yield of the newly emerged individuals of *G. parviflora*.

Keywords. *Galinsoga* spp; weed biology; population regulation; seedling survival.

Introduction

Galinsoga ciliata (Raf) Blake and *G. parviflora* Cav, are sympatric annual herbs of family Asteraceae. Although recently introduced in this country (Babu 1969), they have become important weeds of croplands, jhum fallows and other disturbed habitats in the hill regions of north-east India. Their synchronous and short life cycle (Rai and Tripathi 1983), the appearance of more than one seedling cohort in a single year (Usami 1976) and the ability to successfully invade disturbed habitats make the two weeds ecologically interesting experimental material. In Meghalaya both weeds grow from late March to September as summer annuals. During this period three distinct seedling cohorts emerge at short intervals (Rai and Tripathi 1984). The late emerging seedling cohorts experience competition from the earlier established cohorts of different ages. The present study was carried out to examine the effect of the established cohorts of these weeds on the survival and growth of their newly emerged seedling populations.

Materials and methods

Mature seeds of each species was collected in October 1980 from natural populations occurring on roadsides in Shillong and stored in paper bags in the laboratory where the temperature ranged from 6°C to 20°C. The seeds were germinated in petri dishes by keeping them between two layers of moist filter paper at $20 \pm 2^\circ\text{C}$ in a BOD incubator. Ten 2-day old seedlings were transplanted on 9 April, 4 May and 27 May 1981 in each plastic pot (21 cm diameter containing 4 kg sandy loam soil) to obtain plant populations of different ages. The populations that were 50, 25 and 2 days old on 27 May 1981 had 18, 12 and 2 leaves per plant in *G. ciliata* and 22, 13 and 2 in *G. parviflora*.

In these pots, seeds of each of the two species were sown at the rate of 100 seeds per pot on 27 May 1981. Seeds were also sown in pots where the seedlings were not planted earlier. The experimental design consisted of 7 treatments \times 2 species \times 4 replicates, involving 56 pots which were randomised completely. The treatments are summarized below:

- (i) *G. ciliata* seeds sown in pots devoid of the weed plants (control set).
- (ii) *G. ciliata* seeds sown in pots containing 2-day old *G. ciliata* seedlings.
- (iii) *G. ciliata* seeds sown in pots containing 2-day old *G. parviflora* seedlings.
- (iv) *G. ciliata* seeds sown in pots containing 25-day old *G. ciliata* plants.
- (v) *G. ciliata* seeds sown in pots containing 25-day old *G. parviflora* plants.
- (vi) *G. ciliata* seeds sown in pots containing 50-day old *G. ciliata* plants.
- (vii) *G. ciliata* seeds sown in pots containing 50-day old *G. parviflora* plants.

Similar treatments were established for *G. parviflora*.

The pots were supplied with equal amount of water and kept in a polythene roofed net house (temperature ranged from 29 °C in May to 19 °C in August) on the campus of the School of Life Sciences, North-Eastern Hill University, Shillong (25°34'N, 91°56'E; 1500m altitude).

Seedling emergence in each pot was recorded over a two-week period after which germination practically ceased. The emerged seedlings were marked and their fate was followed at fortnightly intervals until flowering and seeding. The number of survivors and fertile plants per pot and the number of capitula and seeds per reproducing plant and per unit area were estimated on 25 and 26 August 1981. The number of leaves and leaf area per plant were also estimated. After recording the above observations, the plants were separated into root, stem, leaf and capitula and oven-dried at 60 °C for 72 hr and weighed.

Data were analysed through analysis of variance.

3. Results

3.1 Seed germination

Seed germination of the two weeds decreased in the pots containing established seedlings or plants (figure 1). The decrease was more marked when seeds of one species were sown with seedlings or plants of the other species.

3.2 Survivorship of the newly emerged seedlings

Survival of the seedlings was negatively correlated with the age of the established plants or seedlings, and the decrease in survivorship was more when one species was grown with another (figure 1). *G. parviflora* grown with 25-day old plants of *G. ciliata* suffered 80 % mortality which was greater than that observed with 50-day old plants. In general, *G. ciliata* exhibited better survival than *G. parviflora*.

Mortality was heavier during the early stage and decreased later as the seedling populations became older (figure 2). Seedlings of *G. ciliata* experienced mortality throughout the life, while that of *G. parviflora* showed much greater mortality during

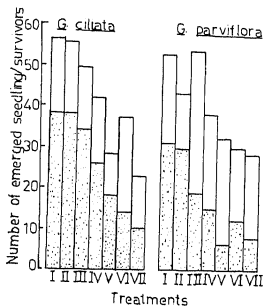


Figure 1. Per pot seedling emergence and survivorship (dotted portions) of *G. ciliata* and *G. parviflora* as affected by the growth of their established plants of different age. Seedling emergence is indicated by the total length of the bar under a given treatment.

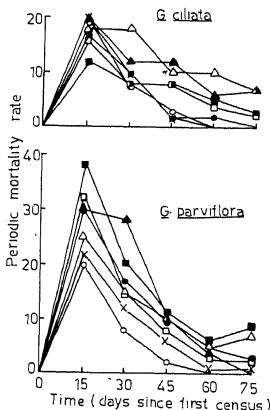


Figure 2. Periodic mortality (%) of seedling cohorts of *G. ciliata* and *G. parviflora* as influenced by the growth of their established plants of different age. Treatments I-VII are respectively (×—×), (○—○), (●—●), (□—□), (■—■), (Δ—Δ), (▲—▲) symbols.

3.3 Leaf production

The number of leaves and leaf area per plant in both species were reduced by

Treatments	<i>G. ciliata</i>	<i>G. parviflora</i>
I	14.4 ± 2.1	18.0 ± 2.9
II	14.5 ± 1.8	18.4 ± 2.6
III	14.5 ± 1.3	16.6 ± 1.9
IV	14.0 ± 1.6	17.0 ± 1.5
V	12.2 ± 0.9	10.0 ± 0.8
VI	12.0 ± 1.0	12.3 ± 0.9
VII	10.4 ± 0.6	12.2 ± 1.0
<i>Response:</i>		
Effect of species	NS	< 0.05
Effect of age	< 0.05	< 0.05
Species × age	NS	< 0.01

NS, not significant

Table 2. Percentage reduction (–) or increase (+) in mean leaf area and biomass per plant of *G. ciliata* and *G. parviflora* caused by their established plants of varying age.

Species	Parameters	Treatments					
		II	III	IV	V	VI	VII
<i>G. ciliata</i>	Leaf area/plant	+3.2	+5.2	–21.9	–32.9	–30.0	–40.4
	Biomass/plant	+7.1	+31.2	–26.0	–6.5	–41.6	–9.8
<i>G. parviflora</i>	Leaf area/plant	+2.8	–24.1	–24.4	–52.7	–33.0	–34.2
	Biomass/plant	–2.3	–2.8	–25.3	–33.7	–9.6	–27.0

cohort reduced the leaf area of the late emerging *G. parviflora* by 53%, while the reduction caused by the 50-day old cohort was only 34% (table 2).

Leaf area per pot also decreased (figure 3) and the magnitude of decrease was greater when one species grew with another. *G. parviflora* was more sensitive to competition from the established cohorts than *G. ciliata*.

3.4 Biomass accumulation

Dry matter yield was also adversely affected by the established cohorts (figure 4). The established cohorts of *G. parviflora* did not cause reduction in yield of late emerged *G. ciliata*, while those of *G. ciliata* did reduce the yield of late emerged *G. parviflora*. Like other growth parameters, biomass of late emerging seedlings of *G. parviflora* was also greatly reduced by 25-day old *G. ciliata* cohort (table 2).

3.5 Reproductive growth

Reproductive performance as indicated by the number of fertile plants per pot and the number of capitula and seeds per plant was reduced by the established cohorts (table 3). Older cohorts caused greater reduction. In contrast to *G. parviflora*, *G. ciliata* grown

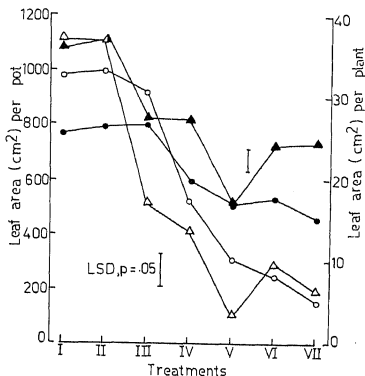


Figure 3. Leaf area of *G. ciliata* and *G. parviflora* seedling cohorts as affected by their established plants of different age. Circles represent *G. ciliata* and triangles *G. parviflora*; open symbols show per pot values and filled symbols per plant values.

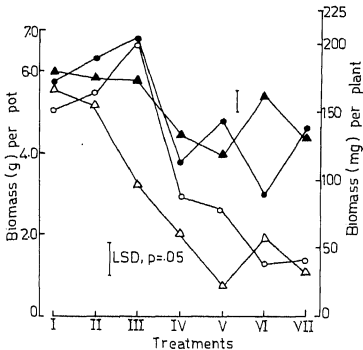


Figure 4. Dry matter yield of seedling cohorts of *G. ciliata* and *G. parviflora* as affected by their established plants of different age. Details of the symbols are the same as given in figure 3.

with established cohort of *G. parviflora* exhibited greater fertility and produced more capitula and seeds than when it was grown with its own established cohorts. *G. parviflora* grown with 25-day old cohorts of *G. ciliata* failed to flower (table 3).

Table 3. Reproductive performance of *G. ciliata* and *G. parviflora* as affected by their established plants of varying age (mean \pm S.E. values are given).

Parameters	Treatments						
	I	II	III	IV	V	VI	VII
<i>G. ciliata</i>							
Percentage of fertile plant	100.0 \pm 0	100.0 \pm 0	100.0 \pm 0	76.9 \pm 4.3	88.8 \pm 5.3	57.1 \pm 3.4	30.0 \pm 3.9
No. of capitula/plant	5.6 \pm 1.0	5.8 \pm 0.9	6.2 \pm 1.2	2.6 \pm 0.8	2.9 \pm 0.3	1.2 \pm 0.4	1.4 \pm 0.3
No. of seeds/plant	91.0 \pm 7.5	95.0 \pm 8.0	100.0 \pm 12.0	37.0 \pm 2.0	41.0 \pm 4.0	17.0 \pm 4.0	19.0 \pm 3.0
<i>G. parviflora</i>							
Percentage of fertile plant	100.0 \pm 0	100.0 \pm 0	100.0 \pm 0	40.0 \pm 3.8	0.0 \pm 0	50.0 \pm 4.3	25.0 \pm 3.8
No. of capitula/plant	3.9 \pm 1.0	4.2 \pm 1.3	3.5 \pm 1.2	1.8 \pm 1.0	0.0 \pm 0	0.9 \pm 0.3	0.4 \pm 0.2
No. of seeds/plant	82.0 \pm 7.5	93.0 \pm 8.2	78.0 \pm 4.9	36.0 \pm 3.8	0.0 \pm 0	16.0 \pm 2.0	8.0 \pm 2.0
Source of variation				Probability			
	Response of <i>G. ciliata</i>			Response of <i>G. parviflora</i>			
	Fertile plant	Capitula	Seeds	Fertile plants	Capitula	Seeds	
Effect of species	NS	NS	NS	< 0.05	< 0.1	< 0.1	
Effect of age	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	< 0.05	
Species \times age	NS	NS	NS	< 0.1	< 0.1	< 0.1	

NS = Not significant

Table 4. Reproductive allocation (% \pm S.E.) of *G. ciliata* and *G. parviflora* as affected by their established plants of varying age.

Treatments	<i>G. ciliata</i>	<i>G. parviflora</i>
I	14.8 \pm 2.1	10.1 \pm 1.2
II	15.3 \pm 1.8	10.3 \pm 1.2
III	17.1 \pm 1.6	8.3 \pm 0.6
IV	7.1 \pm 0.4	3.1 \pm 0.3
V	8.9 \pm 0.5	0.0 \pm 0.0
VI	3.1 \pm 0.2	2.2 \pm 0.2
VII	3.2 \pm 0.2	1.0 \pm 0.2
<i>Response:</i>		
Effect of species	NS	< 0.1
Effect of age	< 0.05	< 0.05
Species \times age	NS	< 0.1

NS, not significant

allocation of *G. parviflora* caused by the established cohorts of *G. ciliata* was greater than that by its own established cohorts. The reduction in reproductive allocation of the late emerging plants was correlated with the age of the established cohorts. However, the 25-day old cohort of *G. ciliata* completely suppressed the flowering in *G. parviflora*.

4. Discussion

were present is in conformity with the findings of Tamm (1956), Putwain and Harper (1970) and Singh (1980), who reported poor germination and seedling survival in established communities. This may be due to non-availability of enough 'safe-sites' (*sensu* Harper *et al* 1965). In another study (Rai and Tripathi 1984), in spite of a large population of seeds in soil, seed germination and seedling establishment of the two species of *Galinsoga* were quite low on the habitats having dense vegetation.

Survival of the newly germinated seedlings of *G. ciliata* and *G. parviflora* was greatly reduced by competition from the established cohorts, and the reduction was often correlated with the age of the cohorts. This is in agreement with the results of Gupta and Tripathi (1979). Both species of *Galinsoga* responded to the competition from older cohorts through mortality and plasticity. Mortality of the two weeds occurs in a different fashion. Whereas in *G. ciliata* mortality was prolonged and occurred throughout the life, *G. parviflora* showed heavy mortality during early stage of life. Relatively greater mortality of the two weeds in interspecific competition agrees with the observations of Friedman (1971) and Yadav and Tripathi (1983) in other plant species. *G. parviflora* experienced maximum mortality when grown with the 25-day old cohort of *G. ciliata*, which was even larger than that observed with the 50-day old cohort. Such a behaviour may probably be attributed to decreased intensity of competition offered by 50-day old plants of *G. ciliata*, which have entered the passive growth phase on account of advanced age. Established cohorts of *G. ciliata* suppressed the growth of both species whilst those of *G. parviflora* could suppress its own individuals only, showing that *G. ciliata* plays a more important role in population regulation of the two weeds. The greater regulatory influence of *G. ciliata* may be accounted for its frequent branching and faster growth producing greater shading in pots (Rai and Tripathi 1983). Thus, the availability of light to the newly germinated seedlings may be curtailed to cause reduction in growth. The relatively little effect of *G. parviflora* on the seedling growth of both species may be linked to its erect habit and open canopy. Seedlings of *G. parviflora* were suppressed more than those of *G. ciliata*, indicating its greater susceptibility to competition from the established cohorts.

Reduced reproductive growth in late emerging plants of the two weeds caused by their established cohorts conforms to the observations of Pemadasa and Amarasinghe (1982) on certain grasses. The reproductive allocation in *G. ciliata* was not reduced to the same extent as in *G. parviflora*, demonstrating that the former species is relatively better adapted to competitive situations than the latter. In general, the reduction in reproductive performance of the late emerged individuals was a function of the age of the established cohorts. However, *G. parviflora* grown with 25-day old cohort of *G. ciliata* showed absence of fertility and the survivors remained in the state of 'resistance to inanition'—a phenomenon described by Chippindale (1948). The results suggest that the established cohorts of *G. ciliata* and *G. parviflora* exercise strong regulatory influence on their newly recruited seedling cohorts through increased mortality and plastic reduction in seed output of the surviving plant, *G. ciliata* having strong suppressive effect on seedlings of both weeds and *G. parviflora* on its own seedlings.

References

- Babu C R 1969 *Galinsoga ciliata* (Raf) Blake (Asteraceae)—a species new to India; *Bull. Bot. Surv. India* **11** 184–185
- Chippindale H G 1948 Resistance to inanition in grass seedlings; *Nature (London)* **161** 65
- Friedman J 1971 The effect of competition by adult *Zygophyllum dumosum* Bioss. on seedlings of *Artemisia herba-alba* Asso. in the Negev desert of Israel; *J. Ecol.* **59** 775–782
- Gupta G P and Tripathi R S 1979 Competition between *Bothriochloa pertusa* (L.) A. Camus and *Dichanthium annulatum* (Forsk) Stapf as modified by their time of emergence in mixture; *Trop. Ecol.* **20** 147–154
- Harper J L, Williams J T and Sagar G R 1965 The behaviour of seeds in soil I. The heterogeneity of soil surfaces and its role in determining the establishment of plants from seeds; *J. Ecol.* **53** 273–286
- Pemadasa M A and Amarasinghe L 1982 The ecology of montane grassland in Sri Lanka V. Interference in populations of four major grasses; *J. Ecol.* **70** 731–744
- Putwain P D and Harper J L 1970 Studies in the dynamics of plant population III. The influence of associated species on populations of *Rumex acetosa* L. and *R. acetosella* L. in grassland; *J. Ecol.* **58** 251–264
- Rai J P N and Tripathi R S 1983 Population regulation of *Galinsoga ciliata* and *G. parviflora*: Effect of sowing pattern, population density and soil moisture and texture; *Weed Res.* **23** 151–163
- Rai J P N and Tripathi R S 1984 Population dynamics of different seedling cohorts of two co-existing annual weeds, *Galinsoga ciliata* and *G. parviflora*, on two contrasting sites; *Acta Oecol. -Oecol. Plant* **5** 357–368
- Singh A 1980 *Studies on population dynamics of Eupatorium odoratum* L., *E. adenophorum* Spreng. and *E. riparium* Regel; Ph.D. Thesis, North-Eastern Hill University, Shillong, India
- Tamm C O 1956 Further observations on the survival and flowering of some perennial herbs; *Oikos* **7** 273–292
- Usami Y 1976 Ecological studies on weeds in mulberry fields 2. Autecology of *Galinsoga parviflora* Cav. *Weed Res. Jpn* **21** 76–80
- Yadav A S and Tripathi R S 1983 The populations of transplanted seedlings of *Eupatorium adenophorum* and *E. riparium* as regulated by their adult plants; *Trop. Ecol.* **24** 201–215

The veinsheath syndrome in Cunoniaceae I *Pancheria* Brongn. & Gris.

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Abstract. Veinlet sheathing of twenty-six species of *Pancheria* is described and illustrated. Within the genus foliar vein sheathing varies from almost completely lacking to abundant, and individual sheath cells show much variation in cell wall composition and structure. The topographical distribution of sheath cells along the vein system, when coupled with the structure of individual sheathing elements, can be utilized to distinguish three anatomical groups of species. Although the evolutionary sequence and ecological significance of the diverse sheath types are at present unresolved, features of vein sheathing appear to be of systematic significance and may provide an additional basis for a better understanding of infrasppecific relationships within this genus.

Keywords. Veinsheath syndrome; *Pancheria*; Cunoniaceae

1. Introduction

There exists considerable anatomical variation with regard to the type and distribution of sheath cells associated with the ultimate leaf venation in the Cunoniaceae (Dickison 1975), suggesting a progressively increasing differentiation of the bundle sheath cells at the vein terminus. An investigation of foliar anatomy was undertaken with a view to record the total range of structural variation, topographical distribution, and potential systematic usefulness of veinsheath elements within the family.

Our initial contribution deals with the endemic New Caledonian genus *Pancheria* Brongn. & Gris. *Pancheria* is composed of ca. 26 species (Guillaumin 1948) that occur in a variety of habitats. They range in growth form between small to medium-sized shrubs or small trees, with a few species assuming the rosette tree form. Leaves are quite diverse in morphology, size, texture, and hairyness.

2. Materials and methods

A total of twenty-six species were examined. Leaf sectors were initially partially cleared by soaking them in 5% sodium hydroxide overnight at 60 °C. Subsequently, they were thoroughly washed in distilled water and flooded with a mixture of trichloroacetic acid and phenol (2:1) for 30 minutes at 60° (Mohan Ram and Nayyar 1978) until they became perfectly transparent (Rao and Naidu 1981). The sectors were dehydrated in an alcohol series and mounted in Canada Balsam without staining. A few pieces were mounted in Lactophenol for camera lucida sketches. The following species of *Pancheria* were studied.

(P); *P. brunshesii* Pampan., Balansa 1070 (F); *P. calophylla* Guill., Balansa 5508 (F); *P. communis* Bak. f., Dickison 181 (NCU); *P. confusa* Guill., Dickison 177 (NCU); *P. elegans* Brongn. & Gris, Dickison 158 (NCU); *P. engleriana* Schltr., Dickison 161 (NCU); *P. ferruginea* Brongn. & Gris, Dickison 165 (NCU); *P. gatopensis* Vieill. ex Guill., Dickison 271 (NCU). *P. heterophylla* Vieill. ex Guill., Vieillard 2252 (P); *P. hirsuta* Vieill. ex Pampan., Dickison 143 (NCU); *P. humboldtiana* Guill., Baumann-Bodenheim 15515 (A); *P. insignis* Schltr., McKee 1106 (A); *P. multijuga* Guill., Baumann-Bodenheim 15357 (Z); *P. obovata* Brongn. & Gris, Baumann-Bodenheim 15012 (Z); *P. phylliraeoides* Brongn. & Gris, ex Guill., Guillaumin & Baumann-Bodenheim 13163 (Z); *P. pinnata* Pampan., McKee 4390 (A); *P. pirifolia* Brongn. & Gris, Guillaumin & Baumann-Bodenheim 13201 (A), 13163 (Z); *P. reticulata* Guill., Guillaumin & Baumann-Bodenheim 11219 (Z); *P. rivularis* Schltr., Hurlimann 1772 (A); *P. robusta* Guill., Dickison 171 (NCU); *P. sebertii* Guill., Guillaumin & Baumann-Bodenheim 10590 (Z), 14444 (A); *P. ternata* Brongn. & Gris, Baumann-Bodenheim 13867 (A). *P. vieillardii* Brongn. & Gris, Balansa 3292 (P).

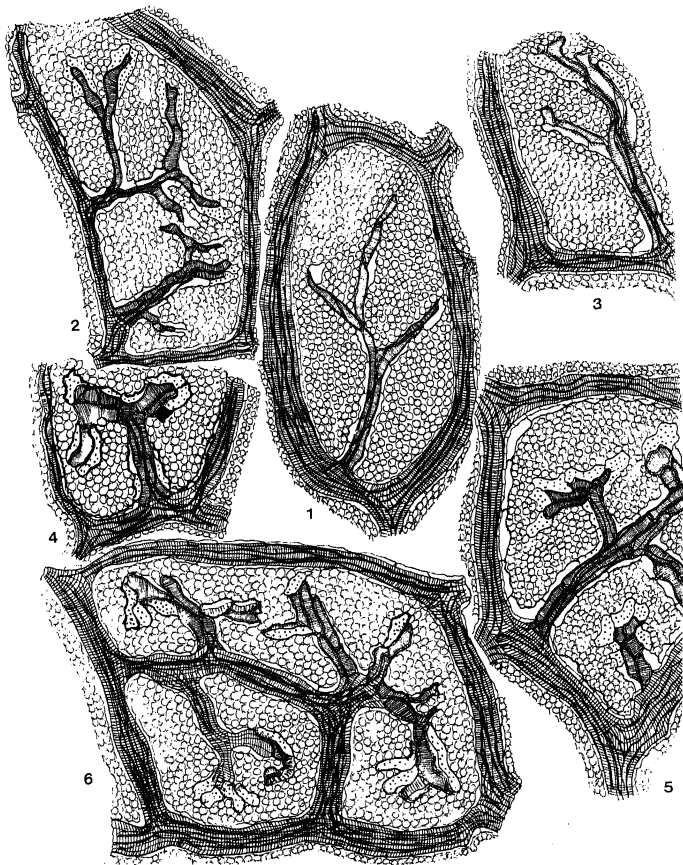
3. Observations

Species of *Pancheria* show three distinct categories of bundle sheathing.

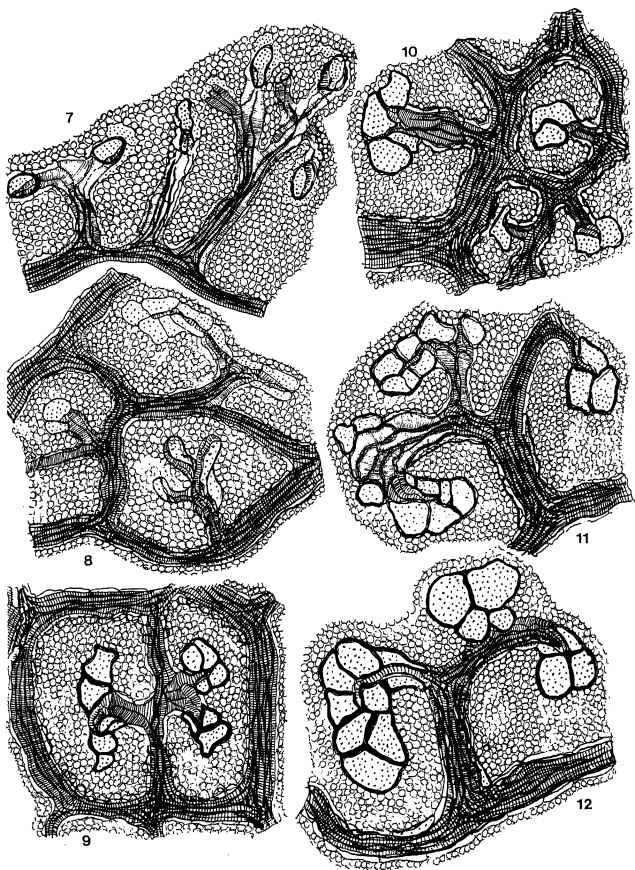
Veinlets provided with sheathing of parenchymatous cells combined with thin to thick-walled, pitted cells at the vein termini (figures 1–6, 13). The bundle sheathing is continuous or occasionally interrupted by imperfectly oblong to cylindrical cells with non- or slightly lignified secondary walls. Vein endings consist of uniseriate or biseriate linear tracheids. In some cases the terminal elements are accompanied by dilated, pitted cells that resemble the elements of the sheath. These features are observed in *P. beauverdiana*, *P. billardieri*, *P. ferruginea*, *P. phylliraeoides*, *P. pinnata*, *P. reticulata*, *P. sebertii*, and *P. ternata*.

Veinlets devoid of sheathing or associated with only a few sheath cells, but possessing a cluster of enlarged, sclerotic, pitted or helicoid cells at the vein endings (figures 7–12, 21–24). Sheathing is either absent or inconspicuous around the vein reticulum. Where present the sheath is composed of uniformly thick-walled parenchymatous cells, and a few sclerotic elements resembling sclereids. Sheathing elements are birefringent and their wall reaction is positive to phloroglucinol-HCL test. Veinlets are terminated by clusters of sclerotic cells of varied sizes and shapes, that range from globose or ovoid to cylindrical. This syndrome characterizes *P. brunshesii*, *P. calophylla*, *P. confusa*, *P. gatopensis*, *P. heterophylla*, *P. hirsuta*, *P. insignis*, *P. obovata*, *P. robusta*, and *P. vieillardii*.

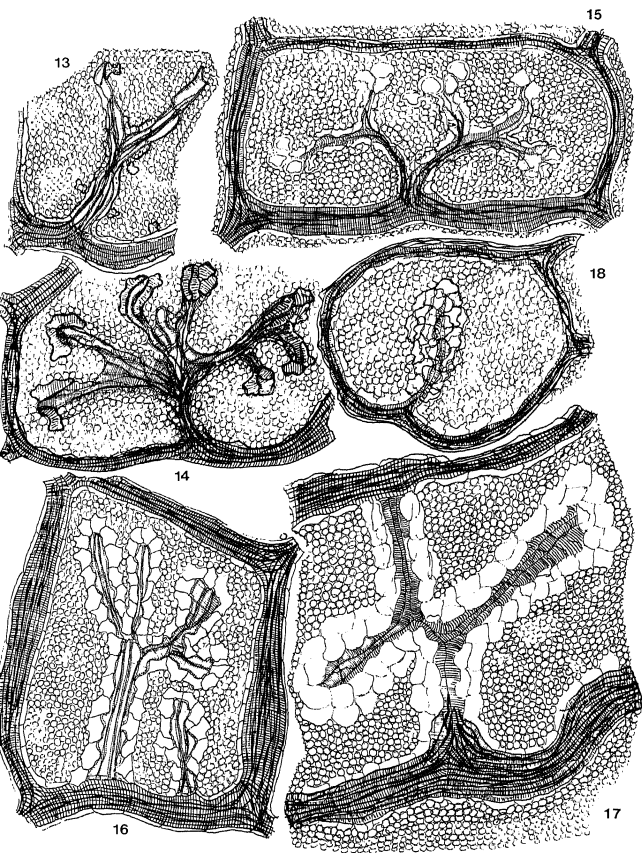
Vein reticula are without prominent sheath cells whereas the bulbous or club-shaped ultimate veinlets are surrounded by a conspicuous sheath consisting of one to a few layers or cells (figures 14–20). The third category, as observed in *P. alaternoides*, *P. communis*, *P. engleriana*, *P. humboldtiana*, *P. multijuga*, *P. pirifolia*, is distinguished by the presence of single, double, or multiple sheath layers of dilated cells around the ultimate veinlets. The sheath cells vary in size and shape although they are characteristically thin-walled, non-pitted and non-lignified. The veinlets are typically bulbous or club-shaped in appearance. They are surrounded by oblong sheath cells



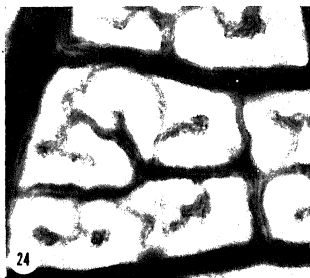
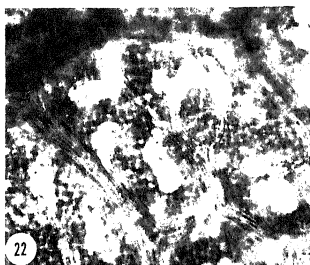
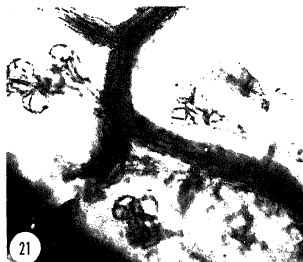
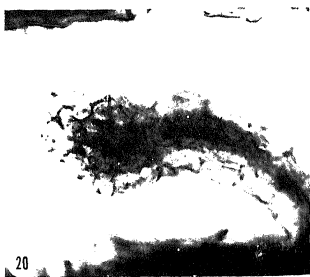
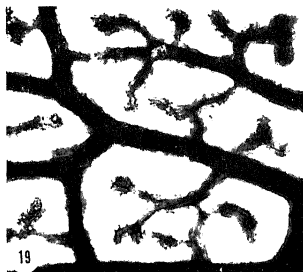
Figures 1-6. Camera lucida drawings of cleared leaves of *Pancheria* showing venet sheathing. All $\times 60$. 1. *P. pinnata* (McKee 4390). 2. *P. ternata* (Baumann-Bodenheim 13867). 3. *P. rivularis* (Hürlimann 1772). 4. *P. ferruginea* (Dickson 165). 5. *P. phylliraeoides* (Guillaumin & Baumann-Bodenheim 13163). 6. *P. reticulata* (Guillaumin & Baumann-Bodenheim 11219).



Figures 7-12. Camera lucida drawings of cleared leaves of *Pancheria* showing veinlet



Figures 13–18. Camera lucida drawings of cleared leaves of *Pancheria* showing veinlet sheathing. All $\times 55$. 13. *P. sebertii* (Guillaumin & Baumann-Bodenheim 10590). 14. *P.*



Figures 19–24. Cleared leaves of *Pancheria* showing veinlet sheathing. 19. *P. communis*

ous sheath encloses the veins of *P. humboldtiana*, (figure 18). The sheath cells associated with the higher order reticula are thin-walled hyaline cells of uniform size and shape, sometimes not clearly distinguished from adjacent mesophyll cells.

4. Discussion

It is evident from the comparative study of twenty-six species of *Pancheria* that the genus is characterized by either the presence, or less frequently the absence, of sheath cells and the occurrence of specialized terminal elements accompanying the ultimate venation of the leaves. Within the genus sheathing varies from essentially lacking to abundant, and individual sheath cells show much variation in cell wall composition and structure in addition to reaction to phloroglucinol and polarized light. The occurrence of multiple layered sheaths composed of thin-walled cells around the veinlets of a few species is particularly striking. Veinlets range between simple, twice or more rarely thrice branched and are composed of single or multiple rows of tracheary elements. Veinlet terminal cells are thin or thick-walled and pitted, and varied in shape. The thicker walled elements have the appearance of terminal sclereids (Metcalf and Chalk 1956; Dickson 1975; Rao and Das 1979). It is unclear whether these terminal idioblasts are derived from procambium or from bundle sheath precursors. None of the species examined possess diffuse foliar sclereids except *P. heterophylla* and *P. sebertii* where sometimes idioblastic subspheroidal sclereids are present near the veinlets.

The topographical distribution of sheath cells along the vein system, when coupled with the structure of individual sheathing elements, can be utilized to distinguish three anatomical groups of species. Each group represents a syndrome of characters with respect to vein sheath orientation and the composition of vein termini that differ markedly from that of the other two groups. Although the evolutionary sequence and ecological significance of the diverse sheath types are at present unresolved, features of vein sheathing appear to be of systematic significance and may provide an additional basis for a better understanding of infraspecific relationships within this genus. Clearly, however, further detailed studies of the species utilizing other parts of plant are required.

Carlquist (1975, 1977, 1980) and Rury and Dickson (1984) have drawn attention to the structural and physiological relationships that exist between the wood and leaf. In some plants the foliage appears to buffer the xylem tissues from prevailing environmental conditions and, therefore, enable more primitive xylem structures to be retained in taxa growing in rather xeric habitats. Despite the fact that some species of *Pancheria* grow in exposed, dry conditions, the wood anatomy of the genus is uniformly primitive and characterized by vessel elements with sclariform perforations (Dickson 1980). This could be explained by the occurrence in these species of very scleromorphic or pubescent foliage that reduces the water demands made by the leaves upon the xylem of the shoot.

Acknowledgements

The second author thanks the many individuals who assisted him in various ways while collecting plants used in the present study. These collections were made possible by the

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References

- Carlquist S 1975 *Ecological strategies of xylem evolution*. (Berkeley, Los Angeles, London: Univ. California Press)
- Carlquist S 1977 Ecological factors in wood evolution: a floristic approach; *Am. J. Bot.* **64** 887–896
- Carlquist S 1980 Anatomy and systematics of Balanopaceae; *Allertonia* **2** 191–246
- Dickison W C 1975 Leaf anatomy of Cunoniaceae; *Bot. J. Linn. Soc.* **71** 275–294
- Dickison W C 1980 Comparative wood anatomy and evolution of the Cunoniaceae; *Allertonia* **2** 281–321
- Guillaumin A 1948 *Flore analytique et synoptique de la Nouvelle-Calédonie-Phanérogames*; (Paris: Office de la Recherche Scientifique Coloniale)
- Metcalfe C R and Chalk L 1957 *Anatomy of the dicotyledons*. Vols. I & II (repr. ed.) (Oxford: Clarendon Press)
- Mohan Ram H Y and Nayyar V 1978 Leaf clearing technique with a wide range of applications. *Proc. Indian Acad. Sci. (Pl. Sci.)* **B87** 125–127
- Rao T A and Das S 1979 Leaf sclereids—occurrence and distribution in the angiosperms; *Bot. Notiser* **132** 319–324
- Rao T A and Naidu T R B 1981 On the epidermal fibre-like sclereids in the two sibling genera of the Poaceae; *Curr. Sci.* **50** 958–959
- Rury P M and Dickison W C 1984 Structural correlations among wood, leaves and plant habit. In *Contemporary problems in plant anatomy* (eds) R A White and W C Dickison (New York and London: Academic Press) pp. 495–540

On the developmental morphology and histochemistry of the galls induced by an agromyzid on the stems of *Pongamia glabra* Vent. (Fabaceae)

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Abstract. The developmental morphology and histochemistry of the solid indehiscent galls of *Pongamia glabra* Vent. are presented. Hyperplasy and hypertrophy of the medullary ray and pith cells causes the separation of the vascular cylinder which contributes to the radial enlargement of the stem. Hyperactivity of acid phosphatase, peroxidase, polyphenol oxidase and an increased level of proteins was detected in the gall tissues and has been discussed in relation to the gall development.

Keywords. Developmental morphology; histochemistry; galls; agromyzid.

1. Introduction

Solid, indehiscent galls on the stems of *Pongamia glabra* induced by an undescribed agromyzid (Diptera) are known (Mani 1973). Since galls in different developmental stages were available, an attempt is made to study the developmental morphology and histochemistry of the gall.

2. Materials and methods

Young, mature and old galls, and normal stems of comparable age, obtained from a private garden in the city of Madras, were fixed in FAA and cut at 7–10 μ m. The sections were stained with safranin-methylene blue. Histochemical localization of proteins, lipids, starch, tannins, and also the enzymes acid phosphatase, peroxidase, and polyphenol oxidase was made using fresh hand-sections of normal and galled stems in different developmental stages. Starch, lipids and proteins were localised following the techniques described by Johansen (1940), Chiffelle and Putt (1951) and Wieme (1959), respectively. Localization of tannin was done by treating fresh sections in Lugol's iodine solution to which a drop of 10 % ammonium hydroxide solution was added that made the tannins to render a bright red colouration. Activities of peroxidase, acid phosphatase and polyphenol oxidase have been localized following the methods of

Observations

Galls (8–15 × 6–12 mm) are relatively soft and green and with maturation, they become harder and develop white patches on the surface (figures 1A, B). The size of the gall is directly proportional to the number of larvae present inside. Mature galls show normally, a single gall chamber extending along the pith region where the larvae feed. Cross sections of the normal stem reveal well-differentiated vascular bundles and parenchymatous cortical, medullary ray and pith cells. Gelatinous fibres occur interspersed in the secondary xylem in both the normal and galled tissues (figures 1E, F). Surrounding the patch of the sclereids parenchyma cells occur in the normal stems, a feature which is absent in normal stems (figures 1G, H). The pith cells lining the gall chamber are hypertrophied and are densely cytoplasmic with prominent nuclei, constituting the nutritive zone. Closer to the inner perimeter of the gall chamber parenchymatous medullary ray cells exhibit considerable meristematic activity lining the xylary patches apart (figures 1D, I). As a result of the feeding by the gall-forming larvae, the pith cells are distorted, and necrosed cells are evident near the feeding sites (figure 1C). As the gall develops, the parenchymatous cells farther away from the nutritive zone redifferentiate into sclereids forming a protective circular ring surrounding the nutritive zone (figure 1D), a common feature in dipteran galls. Further, with the maturing of the galls, some of the parenchyma cells of the pith differentiate into tracheary elements of abnormal morphology establishing vascular communication channels connecting the larval locus with the vascular cylinder of the stem (figure 1J).

Acid phosphatase activity is high in the cortical parenchyma cells, medullary ray cells, phloem parenchyma, the maximum in the nutritive tissues, irrespective of the age of the gall (figures 2A, C). This is significant when compared to the low activity of this enzyme in young and mature normal stem tissues (figure 2B). High peroxidase activity is evident in isolated nutritive tissue cells, medullary ray cells, differentiated parenchymatous cells near the larval locations, and also wall bound in the pith cells (figures 2D–F). In normal stem tissues a poor staining reaction for this enzyme has been evident in the medullary ray cells, pith cells, as well as wall bound in gelatinous cells and pith cells (figures 2G–I). Polyphenol oxidase activity is localised to most of the nutritive cells, and some of the medullary ray cells and cortical parenchyma cells (figures 3A, B). In normal tissues, the concentration of this enzyme is far less, restricted to isolated pith cells and medullary ray cells (figure 3C). Maximum concentration of proteins is evident in the nutritive cells of medullary origin and phloem cells (figure 3D), when compared with the poor reaction in the corresponding tissues of normal counterparts. Starch deposits occur in the pith cells, medullary ray cells and also in isolated cortical parenchyma cells in the normal stem tissues, whereas in the galls they occur in the outer regions of pith, particularly farther away from the nutritive zone (figure 3F). Tannins, in general present a poor profile in gall tissues, while they are completely absent in normal stem tissues. Tannins occur in low concentrations in the developing pith cells, xylem vessels and sclerenchymatous cells away from the nutritive zone of the galls (figure 3E). Reactions for lipids localizations have been studied in normal and in galled tissues.

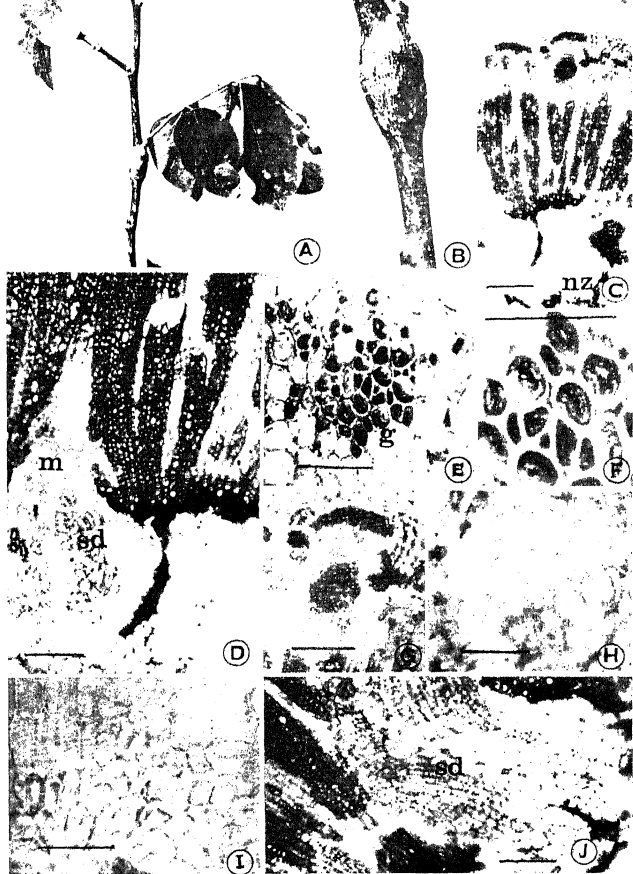


Figure 1. A-B. Stem galls of *Pongamia glabra*. C, D, F-J. Gall stem T.S. E. Normal stem T.S. C. distorted pith cells near the feeding site. D and I. parenchymatous medullary ray cells exhibiting meristematic activity. E and F. Normal and gall stem T.S. respectively showing gelatinous fibres. G and H. parenchyma cells surrounding the patch of sclereids. J. Redifferentiation of pith parenchyma cells into tracheary elements. (g. gelatinous fibres; m- medullary ray cells; sd-sclereid differentiation; nz- nutritive zone) Bar = 100 μ m.

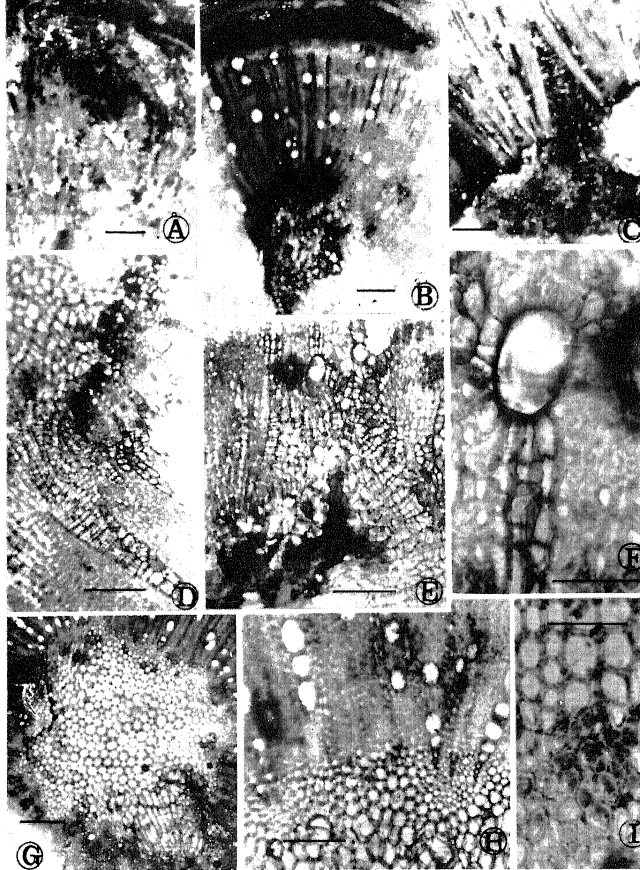


Figure 2. A, C-F. Gall stem T.S. B, G-I, Normal stem T.S. A, B and C. Transverse sections - acid phosphatase localization. D-I. Transverse sections - peroxidase localization. Bar = 100 μ m.

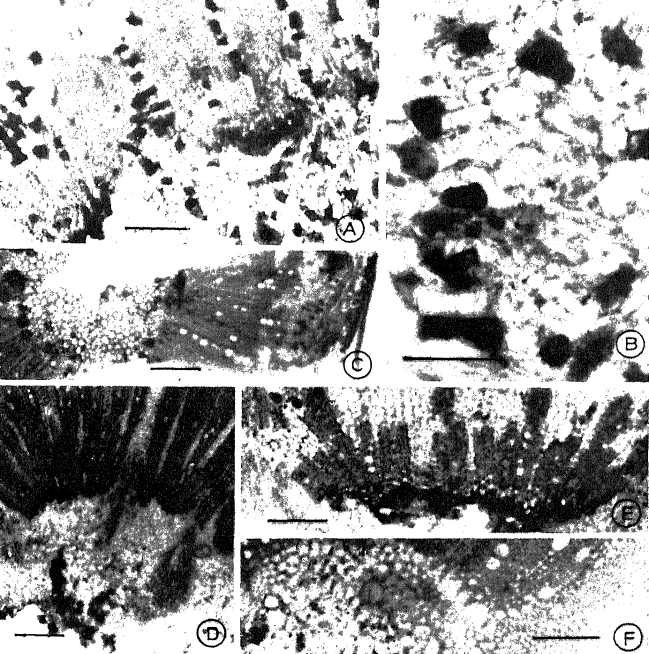


Figure 3. A, B, D-F. Gall stem T.S. C Normal stem T.S. A-C. Localization of polyphenol oxidase. D. protein localization. E. Tannin localization. F. Starch deposits in the outer regions of pith cells. Bar = 100 μ m

4. Discussion

Galls on shoot axis are common in Dicotyledons, the maximum being induced by Diptera, and their structure depends mainly on the position of the larval cavity (Mani 1964). Galls with several larval chambers exhibit considerable structural alterations. In the present investigation, even though more than one larvae per gall occurred in some cases the degree of structural abnormalities were very moderate since the larval feeding was restricted to the medullary region; the influence on the cambial ring was also minimal. This restricted activity of the gall maker provides scope for an overall, but not a detailed, description of the histological changes in the gall stem with special

cation of the shoot axis is mainly contributed by the hyperplasy and hypertrophy of the medullary ray and pith cells, causing the separation of the vascular cylinder, and thus contributing to the radial enlargement of the stem. The proliferation of the cells around the feeding site and in the organization of functionally specialized nutritive cells, involves a reoriented growth polarity in a lateral axis contrary to the normal vertical development. The behaviour of parenchyma cells in *Pongamia* galls appear interesting, in that a two-way action pattern is displayed: (i) the formation of sclereids, and (ii) the formation of tracheary elements, and much discussion has progressed with reference to the histogenetic requirements and developmental coordination as needed in such gall systems (Meyer 1968; Raman and Devadas 1977; Raman and Ananthakrishnan 1979).

A predominant activity of the acid phosphatase in the gall tissues, and the maximum being in the nutritive tissues, comparable to the observations of Christiane (1966) and Bronner and Meyer (1972), appears significant and suggests a higher metabolic activity in cells in the vicinity of feeding spots. Hyperactivity of peroxidase and polyphenol oxidase in specific gall tissues, can be attributed to counter the activity of increased phenols. Wounding, as a result of larval feeding, probably accounts for the higher incidence of protein (Kahl 1982), the maximum noticed in the nutritive tissues alone, and this helps the cecidozoan in its growth and development. Although starch deposits were detected in the pith cells and other tissues of the normal shoot, in the gall tissues their incidence is restricted to medullary ray cells farther away from the nutritive zone. Such an occurrence of starch in cells away from nutritive zone suggests a possible diffusion of soluble saccharides produced by starch hydrolysis towards the nutritive tissues (Bronner 1971, 1975). Tannin distribution is relatively less in *Pongamia* galls and the incidence of tannins in the necrosed pith cells and in some of the xylary elements and sclerenchymatous cells shows their function in this gall being limited.

Acknowledgements

Thanks are due to Prof. T N Ananthakrishnan, for critically going through the manuscript and for comments and to Dr A Raman for his suggestions.

References

- Bronner R 1971 Essais de détermination du pH des tissus nourriciers de quelques galles de cynipides; *C.R. Acad. Sci. Paris* **272** 1680–1682
- Bronner R 1975 Répartition des lipides et de l'amidon dans les tissus nourriciers de quelques galles de cynipides; *Marcellia* **38** 171–177
- Bronner R and Meyer J 1972 Observations histocytologiques comparées de quatre galles 'rudimentaires' en pustule causées par des cecidomyiides; *Marcellia* **37** 77–83
- Chiffelle T L and Putt F A 1951 Propylene and ethylene glycol as solvents for Sudan IV and Black B; *Stain Technol.* **26** 51–56
- Christiane B 1966 Localisation des phosphatases acides dans les galles de cynipides et de cecidomyiides; *C.R. Acad. Sci. Paris* **262** 1437–1440
- Gomori G 1952 *Microscopic Histochemistry – Principles and Practice*, University of Chicago Press, Chicago.
- Isaac W E and Winch N H 1947 Guaiacol-hydrogen peroxide and Benzidine hydrogen peroxide colour reactions in bean (*Phaseolus vulgaris*); *J. Pomol.* **27** 23–27
- Johansen D A 1940 *Plant Microtechnique* (New York: McGraw-Hill)

- Mani M S 1964 *Ecology of Plant galls*. (The Hague: W. Junk) p. 434
- Mani M S 1973 *Plant galls in India*, (New Delhi: Macmillan India) p. 353
- Meyer J 1968 Vascular irrigation dans les galles; *Soc. Bot. Fr. Mem.* **26** 79–97
- Raman A and Devadas C 1977 Morphology, anatomy, and development of the midrib galls on the leaflets of *Lannea coromandelica* (Houtt.) Merr. (Anacardiaceae) caused by *Odinadiplosis odinae* Mani; *Proc. Indian Acad. Sci.* **B86** 156–166
- Raman A and Ananthakrishnan T N 1979 On the morphology of the leaf fold galls of *Maytenus senegalensis* (Lam) Excell. (Celastraceae) induced by *Alocothrips hadrocerus* (Karny) (Thysanoptera: Insecta); *Proc. Indian Acad. Sci.* **B88** 103–107
- Sexton R and Hall J L 1978 Enzyme cytochemistry, In *Electron Microscopy and Cytochemistry of plant cells*, (Ed.) J L Hall (Elsevier North-Holland Biomedical Press, Amsterdam) pp. 63–148
- Weime R J 1959 *Studies on agar electrophoresis* (Arcia uitgraven N.V.) (Brussels and Elsevier, Amsterdam) p. 1965

Cecidogenous *Crotonothrips* (Thysanoptera)—*Memecylon* interactions: Host relations, nutritive tissue, tissue dynamics and cecidogenetic patterns

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Abstract. The host relations of *Crotonothrips* with *Memecylon* on the criterion of gall formation appear interesting. In the light of the morphogenetic courses that the susceptible host organ, the leaf, show, an attempt has been made to assess their functional efficiency in terms of structural adaptations envisaging the organisation of a nutritive zone, and tissue dynamics taking *Memecylon edule*, *Memecylon lushingtonii*, and *Memecylon umbellatum* as models.

Keywords. *Memecylon*; *Crotonothrips*; gall; host relations.

1. Introduction

The cecidogenous association between *Memecylon* and *Crotonothrips* (Phlaeothripidae: Tubulifera) appears striking, since *Crotonothrips gallarum*, *C. coorgensis*, *C. dissimilis*, *C. memecyloicus*, and *C. dantahasta* inducing galls, that range from simple epiphyllous rolls to complex rosettes on *Memecylon* sp., *M. talbotianum*, *M. lawsonii*, *M. lushingtonii*, and *M. edule* respectively, are known from southern India (Ananthakrishnan 1976, 1978). Although this kind of a specialised botanical affinity is frequent among gall-thrips (Ananthakrishnan 1980), the intimate relationship of species of *Crotonothrips* with diverse species of *Memecylon* appears to indicate an important phase in host-relationship patterns of cecidogenous insects, essentially because of their basic trait of organising a specialised nutritive guild in the form of a gall. With this in full view, and also to evaluate comparatively the responses of the host plants to thrips in terms of the development of nutritive tissue and its dynamics in relation to cecidogenesis, an attempt is made here to study three representative types from this gall complex, sampling *Memecylon edule* galls caused by *Crotonothrips dantahasta*, *M. lushingtonii* galls made by *C. memecyloicus*, and *M. umbellatum* galls caused by an undetermined species of *Crotonothrips*. Galls of *M. edule* have been considered here because of their easy availability through the year locally, and the other two represent morphological extremes: *M. umbellatum* galls being simple rolls; *M. lushingtonii* galls being complex rosettes.

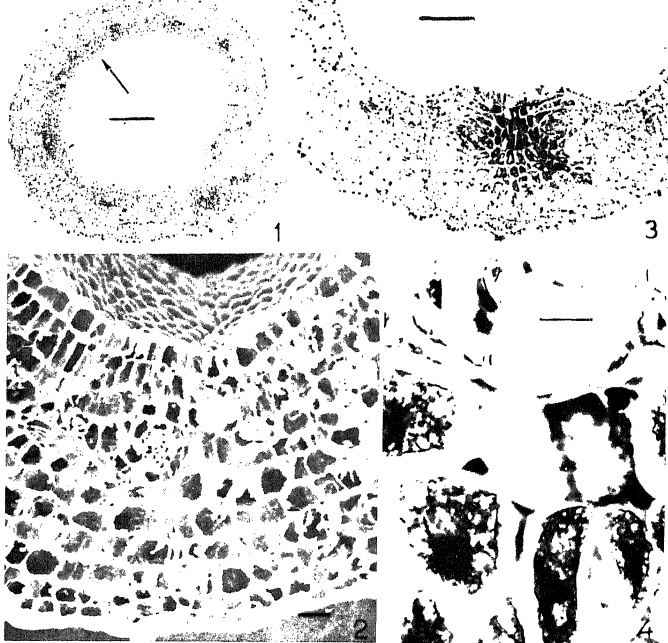
2. Materials and methods

approximate population counts of the inhabiting thrips species, and were fixed in FAA. Through customary methods of dehydration, wax-embedding, cutting (6–8 μm), and staining with methylene blue-safranin combination, the material was prepared for microscopy. Unstained micropreparations were also made for observation under the phase contrast system. Necessary histochemical localisations have been made to confirm some of the observations (details indicated under Observations, wherever necessary). Scanning electron microscopy was also used, following the usual methods of critical point drying (using a Hitachi C. P. Drier), coating with gold, and observing in a Scanning Electron Microscope (Hitachi S-415) system at 15 KvA.

3. Observations

3.1 *Memecylon edule*

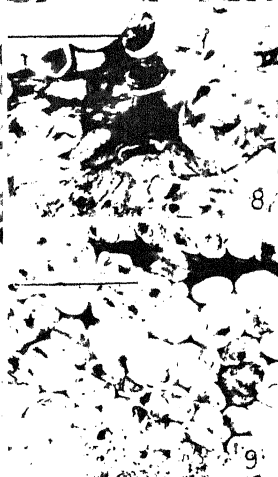
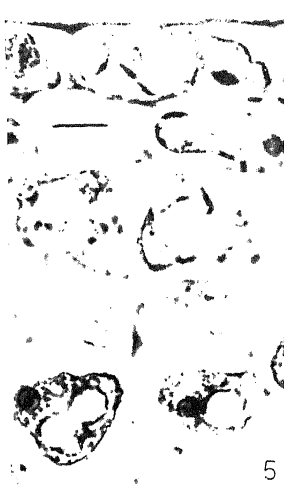
Crotonothrips dantahasta induce epiphyllous roll galls along the developing leaves of *M. edule*. On maturation (about 30 days), the galls become more pronounced with an enormous increase in the thickness of the gall-wall and a number of vertical infoldings, presenting a corrugated appearance. Both laminar halves roll tightly and independently on themselves towards the midrib. In young galls (2–3 days) thrips feed on specific areas along the upper side of the lamina closer to vascular traces (figures 1 and 2). The initial feeding impact involves the rupturing of 1–2 epidermal cells, and the resultant wounding stimulates a few surrounding epidermal cells and the immediate subjacent cells of the differentiating mesophyll to become metaplastic (figure 3); these cells show dense cytoplasm, characteristic of nutritive cells. The differentiation of these functionally specialised nutritive cells is of characteristic pattern that they are localised, involving only a few epidermal cells and 2–3 layers of cells lying immediately below (figure 4). These cells occurring along the direct line of feeding impact of thrips show abundant cytoplasm with numerous agglomerating vacuoles, and hypertrophied nuclei and nucleoli. The walls of these cells are abnormally and unevenly thickened with many irregular infoldings that occasionally show branching as well (figure 5). They do not appear to be of lignin (phloroglucinol test; Johansen 1940); the layers of these walls are shiny and crystalline under normal (figure 14) and phase contrast (figure 15) microscopy, but display characteristic striations and layering in their organisational patterns (figure 17). Not only hypertrophy and hyperplasia are well-manifest in the gall regions of the lamina, but also the total inhibition of differentiation of a dorsi-ventral leaf with 2 layers of palisade tissue and 5 layers of spongy mesophyll. The cells coming directly under the feeding stimulus of thrips show hypertrophy in the vertical axis, and these appear to arise from the primordial palisadic and epidermal cells. The succeeding layers of cells (5th–9th layers) of the upper mesophyll origin are hypertrophied more or less isodiametrically and display meristematism (figure 6). At this stage of gall development, the underlying 4th and 5th layers of cells show greater cytoplasmic specialisation than the cells lying above, suggesting the extension of the nutritive function to the underlying areas of the differentiating blade (figure 5). The lower spongy mesophyll contribute to the two layers of the lower gall lamina, that retain more or less their normal contour. Interestingly, the wall thickness of these cells is varying that the epidermal cells (1st layer) show 3–4 μm thickness; the second layer of nutritive



Figures 1–4. *Memecylon edule*; young gall (2–3 days old). 1. T. S. Nutritive tissue (arrow) (bar = 200 μm). 2. Stereoscan profile (bar = 100 μm). 3. Differentiating nutritive tissue (bar = 100 μm). 4. Feeding injury and nutritive tissue—enlarged (bar = 10 μm).

cells show 4–5 μm thickness while the third layer, with a remarkable drop to 1–2 μm , that gradually increases from the 4th layer onwards to 4 μm till the lower epidermis. In spite of this specific pattern in the thickness of wall in the nutritive and other mesophyll tissues nearer the feeding area, increase in wall thickness seems to be a general alteration in the morphogenetic gradient of the gall-susceptible leaf. This appears significant primarily because abnormally thick cell walls are evident even in non-gall regions of the leaf (figure 19), that show no other specific change except this.

The division patterns and the hypertrophy initiated by the feeding stimulus of thrips are so very characteristic that active division centres radiate in a vertical line till the mid-region of the gall mesophyll; at this point, this line takes a dialatory course that shows a 'fork'ing pattern extending upto the lower epidermis (figure 7). The neighbouring areas of this active meristem line are supported on either side (as in transverse sections) by



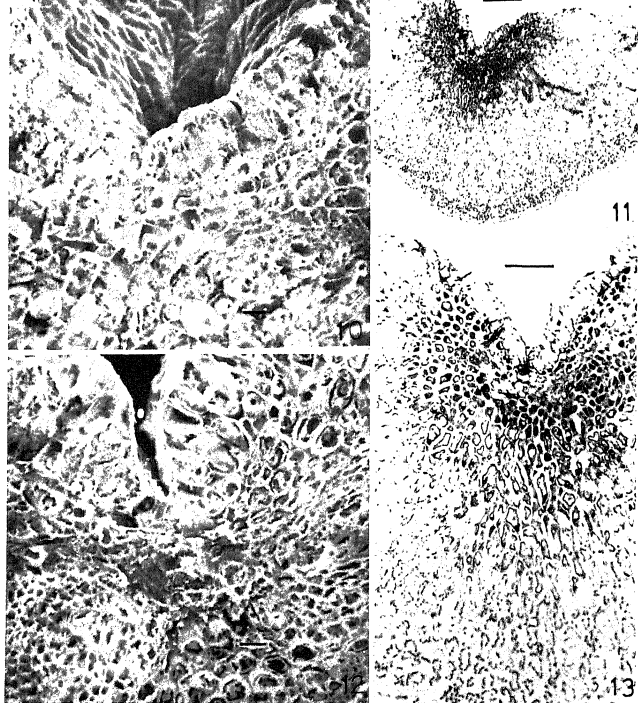
hypophyll or mesophyll cells as well as of those in the farther regions of the gall mesophyll, i.e., beneath the 'fork'. The further growth of the gall is in conformity to this initial morphogenetic canalisation as regulated by the dissimination of the cecidogenetic gradient that originates at the feeding point—a region of constant injury and irritation. A weak but well-coordinated meristem gradient operates around the feeding site, contributing cells for upward growth, which is a distinct feature of this gall.

With ageing (6–9 days), the nutritive cells accumulate flavonolic and tanniferous products as crystalline inclusions distributed especially along the tonoplast (tested by Lugol's iodine test and by the techniques of Mace and Howell (1974) for condensed tannin precursors); the galls attain an overall thickness of about 20 layers of cells with the nutritive tissue localised to 6–8 layers of cells from the upper epidermis. Simultaneously, around the feeding site, the upper mesophyll parenchyma and the upper epidermal cells display meristematic activity that contribute cells, enabling an upward growth of the gall tissue (figures 10, 11). At this stage, the nutritive cells of upper epidermal and palisadic origin continue to expand in the vertical axis, while the primordial palisadic cells show sporadic anticlinal divisions; very curiously, the nutritive (epidermal) cells show periclinal divisions contributing to the lateral, circumferential growth around the feeding site (figures 10, 12, 13). Although moderate division activity is evident along the cells of the upper mesophyll, the cells show enlargement in the tangential plane facilitating the further in-rolling of the lamina. The cells lying closer to the lower epidermis show anticlinal and periclinal divisions as well as stretching in the horizontal axis. Old galls (20–22 days) show an enormous increase in the tanniferous products that almost occlude the cells.

Necrosis, an important cecidogenetic event in thrips gall systems, shows interesting patterns in its incidence. When feeding process is restricted to laminar areas and not directly on vascular regions, necrosed cells extend from upper epidermal cells to a few layers of mesophyll tissue below, extending in a vertical plane (figure 8). On the other hand, when feeding is restricted to vascular regions, necrosis of cells occurs along the upper-most layers of the mesophyll, excluding the epidermal cells, and extends in a horizontal axis (figure 9). This kind of a differential behaviour of the tissue of the same host organ appears unique.

Another outstanding feature of the nutritive tissue of the galls of *M. edule* is the development of a number peg-like ingrowths along the inner side of the wall that extend into the cytoplasm (figure 18), besides the thickening of the cell wall (figure 16). These ingrowths appear to be callosic and show compact distribution and close approximation to one another throughout the inner surface of the cell wall. Although the general thickening of the wall shows a varying profile, the intensity of the development and distribution of these wall ingrowths tends to decline towards the lower epidermis. In other words, these wall ingrowths are characteristic of the nutritive tissue, composed of the upper epidermal and a few subjacent mesophyll cells.

With the initiation of galls, the stomatal areas of the differentiating leaves show interesting responses. In very young galls (2–3 days) the epidermal cells closer to the stomatal pore show proliferation (figures 20, 21), though localised, thereby the stomata are lifted-up (figure 22). With maturation of the galls, the guard cells of the stomatal pores located at the summits of the elevated areas, appear to have lost their characteristic profile and present themselves to be mere circular openings (figure 23). It is also apparent that these stomata have lost their basic function of opening and closing.

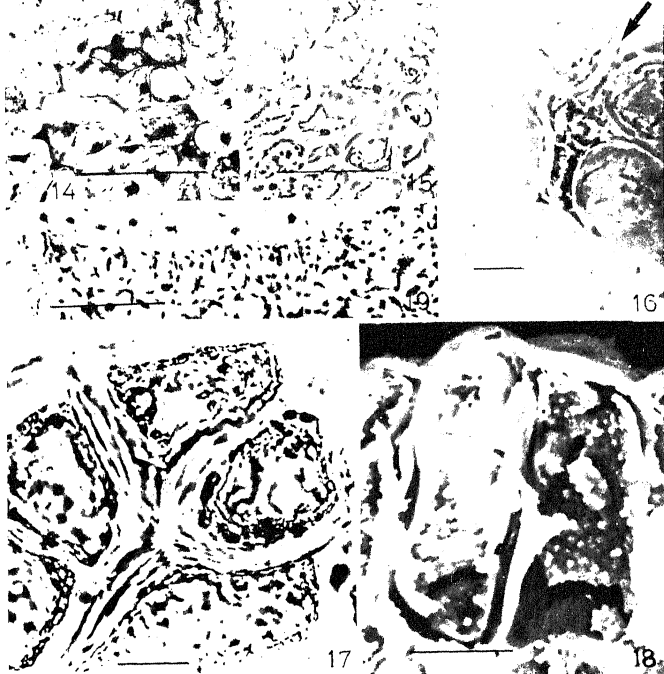


Figures 10–13. *Memecylon edule*; mature gall (5–7 days old). **10.** Stereoscan profile of the feeding zone showing circumferential growth (bar = 100 μ m). **11.** Developing nutritive tissue (T.S.) (bar = 100 μ m). **12.** Stereoscan profile—nutritive tissue (bar = 100 μ m). **13.** Same stage as in figure 7 (T.S.) (bar = 100 μ m).

proliferation are repeated in the same way as they normally would do on the upper side (figures 24,25).

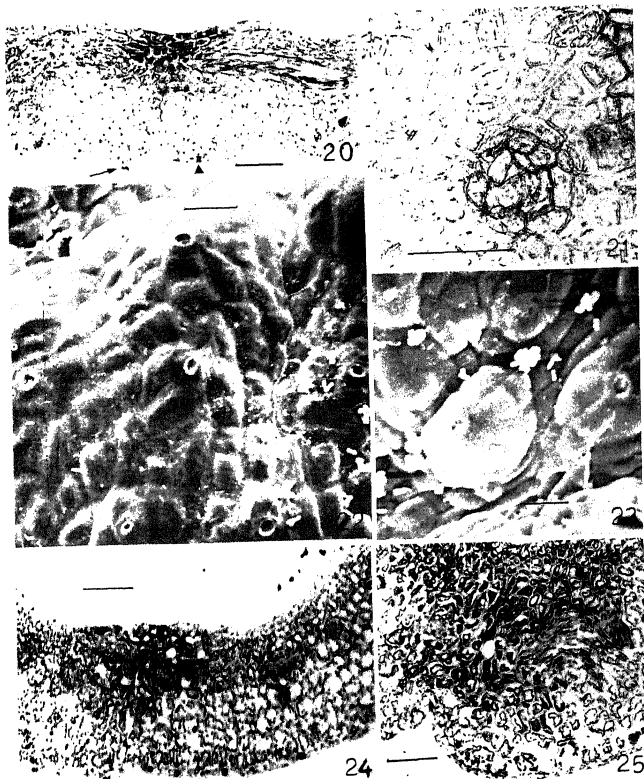
3.2 *Memecylon lushingtonii*

Crotonothrips memecylonicus induces more complex galls on the leaves of *M. lushingtonii* than those of *M. edule*. The galls show greater morphological complexity



Figures 14-19. *Memecylon edule* gall (5-7 days old). 14 and 15. Thickened cell walls of nutritive cells (bar = 50 μ m). 16. Enlarged view of the cell wall nature closer to the feeding area (arrow) (bar = 100 μ m) (phase contrast microscopy). 17. Macerated upper mesophyll cells closer to the feeding zone showing the cell wall nature (cf. striated nature) (bar = 10 μ m) (phase contrast microscopy). 18. Stereoscan profile of the nutritive (epidermal) cells showing peg-like wall ingrowths (bar = 10 μ m). 19. Thickened cell walls along the mesophyll regions of non-gall areas (bar = 100 μ m).

in terms of rugosity and corrugations that the linear profile of the involved leaf is completely lost in the galling process, and the galls are characterised by a rosette-like appearance; interestingly, fusion of galled portions of the two oppositely placed leaves occurs, though infrequently, displaying a tendency to form 'closed' galls with a centrally placed insect chamber—an aspect extremely rare among thysanopterocecidia. Although, in principle, the galls are epiphyllous rolls, the intensity of rolling and the development of vertical partitions is great that the galls are unique



Figures 20–25. *Memecylon edule*. 20. Young gall (T.S.) showing hypertrophy of lower mesophyll and elevation of stomatal pores (arrow) (bar = 100 μ m). 21. Same as in figure 20; surface view (bar = 100 μ m). 22. Stereoscan view of the lower epidermis of young gall (bar = 100 μ m). 23. Same as in figure 22; old gall (bar = 100 μ m). 24. Old gall (20 days old) showing the accumulation of phenolic material in the erstwhile nutritive tissue (bar = 100 μ m). 25. Old gall; induction along the lower epidermis (bar = 100 μ m).



Figures 26–30. *Memecylon lushingtonii*; young gall (1–5 days old). 26. Nutritive tissue (arrow) (bar = 200 μ m). 27. Feeding zone (bar = 100 μ m). 28. Differentiating nutritive tissue (bar = 100 μ m). 29. Nutritive (epidermal) cell (bar = 10 μ m). 30. Nutritive (lower mesophyll) cell of 5 days old gall (cf. wall thickening).

identical morphogenetic processes as described for the galls of *M. edule*; but, significantly, a shallowing of the gall-lamina is evident in response to thrips feeding, besides organising the nutritive tissue by the metaplasia of the epidermal and subepidermal layers at specific areas (figure 28). The epidermal cells coming directly

appears nearly 6 times more than the hypertrophy level of the identical tissue in the galls of *M. edule*. The free regions of the nutritive (epidermal) cells along the adaxial leaf surface show profound cytoplasmic intensity, suggesting an active metabolic status (figure 29).

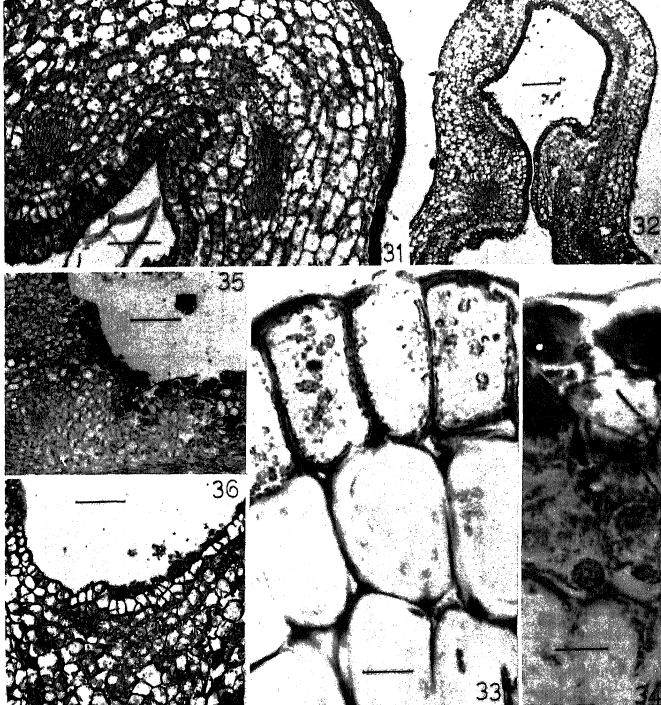
Within 4–5 days of galling, the nutritive area spreads to subjacent layers of the mesophyll as well, and at this stage, the nutritive cells (epidermal and upper mesophyll cells) begin to accumulate phenolic products as crystalline inclusions (figure 30). Although the accumulation of phenolic products is similar to the galls of *M. edule*, the time of initiation of this process appears within 5 days of gall initiation, which is much earlier than that of the galls of *M. edule*.

In spite of the basic processes of gall formation being similar to *M. edule*, the cecidogenetic gradient is distinctly of a specific pattern that proceeds as a straight line (as in transverse sections) up to the lower epidermis. The shallowing of the feeding area appears significant, supported by meristematic activity, though isolated, in the mesophyll regions closer to the upper and lower epidermises, on either flank of the feeding zone (figure 31). Among these, the division centres along the lower gall-line are more active, highlighting the major morphogenetic difference between the galls of *M. lushingtonii* and *M. edule*. Following the achievement of growth along the lower gall-line, the lateral (as in transverse sections) meristems in the upper zones, closer to this feeding areas become more active by adding cells to the formation of the pouch (figure 32). During late stages of gall ontogeny (20–22 days), the gall regions are enormously thickened with up to 50 layers of gall mesophyll with the characteristic shallowing of the gall-lamina, thus facilitating the formation of numerous pouches in the gall-system; this contributes to the profound increase in the surface area of galls, thereby providing a large nutritive area well-within the limited leaf-area.

With maturation, galls of *M. lushingtonii* too show abnormal wall thickenings with many warty wall ingrowths restricted to areas of nutritive cells (figure 33). Accumulation of tanniniferous material is yet another feature that characterises ageing of galls (figure 34), and the nutritive cells in particular that get occluded densely with phenolic material (figures 35, 36, 39).

Old galls (18–20 days) show peculiar cellular behaviour, that the cells of the lower mesophyll layers (derivatives of the primordial spongy mesophyll cells) retain their normal morphology (under galled conditions), while the upper epidermal cells and the subjacent layers of the mesophyll (the erstwhile nutritive cells) show stretching along the horizontal axis, and develop, as a result, large intercellular spaces (figure 37). This appears significant, and is obviously due to dehydration and subcellular molarity changes within gall systems, particularly in the upper layers closer to the insect chamber, coordinating with the ceasing of the feeding stimulus. This facilitates the unrolling of the laminae that have rolled-in tightly, thus enabling the easier migration of thrips populations built-up within the galls.

Rarely, galls of *M. lushingtonii* show meristematic activity in the circumferential area of the feeding spot of young galls, which results in the development of covering 'lip-like' growths enclosing a large insect cavity (figure 38). Besides this, some of the other salient aspects that appear interesting in these galls are: (i) development of nutritive cells in



Figures 31–36. *Memecylon lushingtonii*. 31. Differentiating gall-leaf (T.S.) (1–5 days old) showing the shallowing of the lamina. Proliferation evident along the upper mesophyll (bar = 100 μ m). 32. Mature gall (T.S.) (5–10 days old) with a well-developed, centrally placed insect chamber (bar = 200 μ m). 33. Mature gall—nutritive tissue (bar = 10 μ m). 34. Old gall (20 days old)—nutritive tissue (cf. upper epidermal cells accumulate tannin) (bar = 10 μ m). 35. Old gall (25 days old); intense accumulation of tannin material (bar = 100 μ m). 36. Old gall—region closer to erstwhile nutritive tissue; proliferation and emptying of cells evident (bar = 100 μ m).

3.3 *Memecylon umbellatum*

Crotonothrips sp. induces epiphyllous roll galls on the leaves of *M. umbellatum*. Compared to *M. edule* and *M. lushingtonii* galls, these galls are very simple, displaying

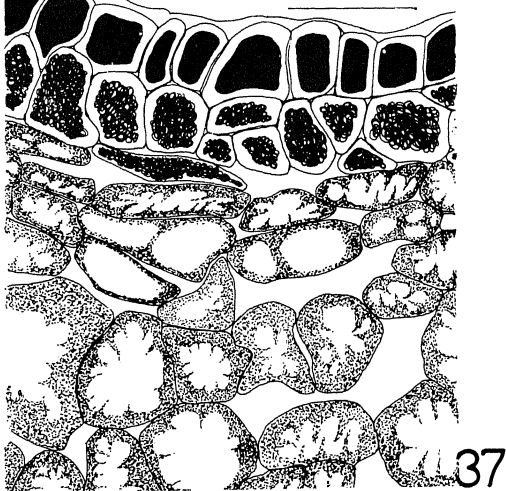
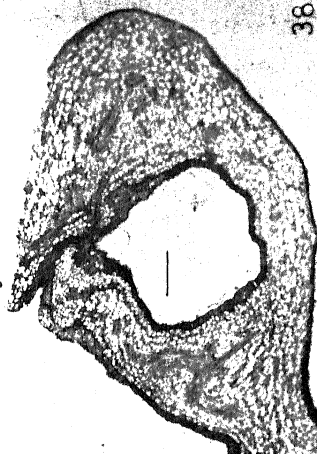
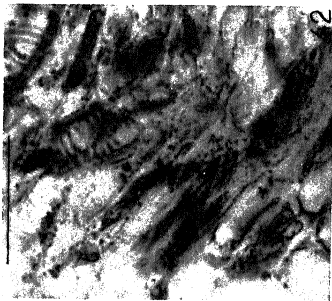


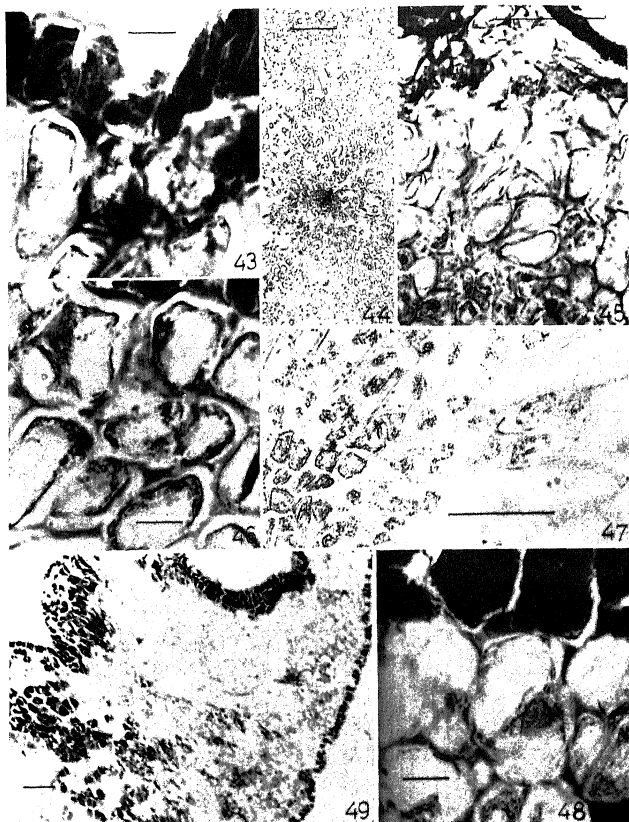
Figure 37. Old gall of *Memecylon lushingtonii*; some of the mesophyll cells showing lateral expansion and development of intercellular spaces.

Interestingly, the galls of *M. umbellatum* are identical to the galls of *M. edule* in their developmental patterns, although the proliferation pattern, supportive hypertrophy, organisation of the nutritive tissue (figures 43, 45, 46, 48) are evident in a minified scale. The circumferential growth around the feeding site is similar to that of *M. edule*. Investigations on the epidermises of differentiating galls show moderate proliferation around the feeding site (figure 44), while with maturation, the epidermal cells, a little away from the puncture area, that correspond to the circumferential growth area, show enormous hypertrophy to a magnitude of 14–15 times with normal division activity (figure 47). The hypertrophy pattern is less-manifest in regions closer to the puncture area and more intense at regions away, showing a sequential increase.

Old galls (20–25 days old) are characterised by the accumulation of tannin material in the nutritive cells that gradually extends to the deeper mesophyll (figure 49), similar to

Figures 38–42. *Memecylon lushingtonii*. 38. Mature gall showing tendency to develop labial covering growth (bar = 200 μ m). 39. Old galls—accumulation of tanniferous and flavanolic material in the nutritive tissue (bar = 50 μ m). 40. Mature gall—nutritive tissue closer to vascular strands (bar = 50 μ m). 41. Normal leaf—sclereids (bar = 50 μ m). 42. Gall (5–10 days old); proliferation of interfascicular parenchyma and transformation into tracheary elements (bar = 50 μ m).





Figures 43–49. *Memecylon umbellatum*. 43. Young gall (1–3 days old)—feeding damage and initiation of nutritive cells (bar = 10 μ m). 44. Young gall; surface view of the circumferential growth around the feeding site (radial pattern of epidermal proliferation) (bar = 100 μ m). 45. Young gall—lateral aspects of the feeding area showing proliferation (bar = 50 μ m). 46. Mature gall (5–8 days old); nutritive tissue (bar = 10 μ m). 47. Mature gall; surface view of the epidermal hypertrophy (bar = 50 μ m). 48. Mature gall (8–10 days old); cells near the nutritive area (wall thickening is less intense) (bar = 10 μ m). 49. Old gall (20 days old) (bar = 100 μ m).

the other two galls in the like mesophyll areas, no significant hypertrophy and development of intercellular spaces is evident as in the galls of *M. lushingtonii*.

4. Discussion

By preferring the leaves of *Memecylon*, species of *Crotonothrips* have, in principle, a fundamentally identical cecidogenetic behaviour; yet, the developmental patterns in terms of specific responses of the host plants are distinct. Based on the degree of complexity, *umbellatum* galls appear to be the simplest of the three, showing a simple external form and developmental events, while the galls of *lushingtonii* present themselves to be of the complex type in form, organisation, and development; tendency to develop covering 'lip-like' growth as well as closed galls, a character more-frequent among the galls of Homoptera and Diptera, appears important. Galls of *edule* provide sufficient scope to be recognised as an intermediate type.

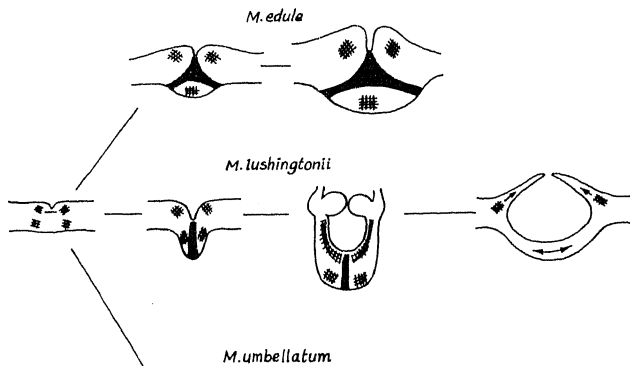
Gall induction by thrips is a specific, population-regulated phenomenon (Ananthakrishnan 1984; Raman and Ananthakrishnan 1984). *Crotonothrips*-*Memecylon* interactions indicate a successful interlinking of the collective feeding effort of the building populations of thrips species with the responses of the host in terms of developmental adjustments, thus rendering each gall system as a functionally effective one. For instance, with ageing, galls of *M. lushingtonii* show highly specialised patterns of tissue behaviour that lead to the loosening of the compact system enabling the easier migration of *Crotonothrips memecyonicus*. Interestingly, this kind of a host behaviour is lacking in the galls of *edule* and *umbellatum*, wherein the systems are less compactly in-rolled. Although the precise physiological nature of the tissue involved is not known, the functional moderation as evident in the structural adjustments of *M. lushingtonii* galls, facilitating the temporal expansion along the horizontal axis of particular tissue is unique.

One of the basic and major demands of insect-induced gall systems is to offer nutritional facility to the gall making organisms. Galls of *edule*, *lushingtonii*, and *umbellatum* provide excellent examples for this cecidogenetic requirement, as a highly specialised nutritive tissue is initiated in these galls, firstly in response to the feeding stimulus, and secondly to the consequent injury and irritation. A purposeful combination of these enables the primordial host tissue to turn metaplastic and display signs of enlargement and occasional proliferation and thus become tissues of special nature with abundant food reserves (Raman and Ananthakrishnan 1983; Gopinathan 1984). Significantly, this tissue shows a series of specialised responses in conjunction with the process of ageing. Initially, the nutritive cells accumulate more and more of cytoplasmic reserves, and within about 7 days, tanniniferous material appear indicating the gradual transformation of this tissue into a non-functional one. But the spread of this subcellular specialisation to the lower mesophyll cells, concurrently with the accumulation of flavanolic and tanniniferous material in the upper mesophyll cells suggests that thrips are able to obtain a continuous supply of nutrition throughout their period of stay within gall systems. Intense accumulation of the phenolic substances in the nutritive cells upon ageing and ultimately senescence, that takes about 25 days in all the three gall systems, synchronises with the general pattern of life-cycle duration of species of *Crotonothrips*.

Histogenetic studies describe how a shift in the direction of division activity is

initiated during cecidogenesis. These also underline the nature of the feeding stimulus that initiates and directs 'new' courses of differentiation. Although growth in terms of hypertrophy and hyperplasia is localised to specific areas of the gall-susceptible leaf, there is an overall impact evident in the total leaf: (i) normal differentiation course is inhibited, thus the whole leaf retains tissues in their primordial profile; (ii) abnormal wall thickenings develop throughout the blade; and (iii) gall-susceptible leaf rolls on itself, although the intensity of rolling varies with species.

Investigations on the growth processes shed considerable light on the tissue behaviour as altered in the cecidogenetic system. Under the general circumstances that the host organ is a leaf, the gall susceptible hosts belong to the genus *Memecylon*, and the gall inducing agents belong to the genus *Crotonothrips*, the gall formation process and the end-products display a highly varied and eventful course of differentiation and growth. This aspect becomes all the more significant essentially because the localised effect of the reaction processes underscores the need to modify the innate rules that govern the orientation patterns of cell division and hypertrophy, so that the tissue geometry, and in effect, the organ symmetry are maintained. Alteration in the course of morphogenetic events to neutralise the stress created by the cecidozoa is obvious by the creation of new, active division centres at specific localities of the organ, and the rest of the system being supported by activities such as cell enlargement, and subcellular adjustments that include wall ingrowths and thickenings, as well as cytoplasmic specialisation as evident in the nutritive cells. Interestingly, the stress areas show varying profiles in the analysed gall systems: *edule* and *umbellatum* galls show an inverted 'Y' pattern, while *lushingtonii* system shows an 'I' pattern, which necessarily predict the further course of morphogenetic events to achieve the ultimate gall-form



feeding site, and the 'Y' course indicates the shallowing of the gall lamina, although in either case, the basic objective of the gall system seems to provide scope for the development of vertical partitions in the rolled leaf, thereby increasing the nutritive surface area well within the limited leaf area. The 'I' and 'Y' patterns of stress situations also indicate very interesting cellular behaviour as well, wherein each constituent cell is moderately hypertrophied in the vertical axis, corresponding to the flow pattern of the cecidogenetic gradient. On the whole, as evident in transverse sections, these gall systems do not sacrifice their bilaterally symmetrical organisational pattern, but broadly, there is an overall exaggeration in cell dimensions at particular areas, and more subtly, there are a number of specific developments that contribute to the spatial organisation of a leaf as a compensated system of a gall suiting the needs of an alien genome.

Every cell participating in cecidogenesis shows a distinct pattern of hypertrophy; though differing in magnitude and intensity, each cell largely conforms to the type of the primordial tissue from which it has originated: cells of palisadic origin show hypertrophy in the vertical axis; cells of spongy mesophyll origin show isodiametric hypertrophy. This tissue behaviour suggests that thrips are able to destabilise the normal polarity gradient only partly. Similarly, the cells of the upper epidermis of *edule* and *umbellatum* show profound meristematic activity enabling the upward circumferential growth; on the other hand, proliferation of the upper epidermis in the galls of *lushingtonii* is minimal, primarily because the galling process involves a greater developmental stress towards shallowing. While such is the morphogenetic course involving a significant divergence in the behavioural trends the upper epidermal and associated mesophyll cells, the lower epidermal and allied mesophyll cells display identical behavioural patterns in all the three species, that the stomatal areas and subjacent mesophyll cells show hypertrophy and proliferation to such an extent that the stomatal pores are elevated from the normal levels indicating the possible development of a water stress. The loss of the contour of guard cells, and the pores remaining open throughout, suggest the functional status of these in aiding the transpirational processes to get over the water stress developed from within, probably as a consequence of gall development. Further, the modification of form of these stomatal pores indicate the establishment of new polarity axes and new courses of differentiation at regions farther away from the stimulus area. Besides these behavioural adjustments in the tissue systems, the development of wall thickening, that shows varying profiles, appears interesting. Probably because the intensity of thickening increases with ageing in cells, thus reducing the surface area, these cells develop peg-like ingrowths that enable an increase in the surface area. Higher rates of incidence of this subcellular differentiation nearer the feeding site and a gradual decrease towards the lower mesophyll support this contention. In all the three species, differentiation of tracheary elements by the hyperplasia of interfascicular parenchyma as well as the total inhibition of sclereid differentiation are evident; the latter becomes extremely important as normal leaves of all the species of *Memecylon* are characterised by the incidence of different kinds of sclereids (Rao 1957). A positive reaction of the gall tissue showing the induction of tracheary elements and a simultaneous negative response of inhibition of the formation sclereids support the idea that cytodifferentiation stems from epigenetic modifications (Davidson 1968; Maresquelle 1980) as these appear as directed changes, although the question relating to the reversibility potential of these

behaviour involving structural and functional changes create difficulties in establishing a causal relationship with the stimulus from the gall maker, since the activity patterns towards changes in polarity axes are moderate in some areas, but strong in some other areas.

Acknowledgement

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References

- Ananthakrishnan T N 1976 New gall thrips of the genus *Crotonothrips* (Thysanoptera); *Orient. Insects* **10** 411–419
- Ananthakrishnan T N 1978 Thrips galls and gall thrips; *Zool. surv. India, Tech. Monogr.* **1** 1–69
- Ananthakrishnan T N 1980 Thrips—plant gall association with special reference to patterns of gall diversity in relation to varying thrips populations; *Proc. Indian Natl. Sci. Acad.* **B47** 41–46
- Ananthakrishnan T N 1984 Adaptive strategies in cecidogenous insects; In *Biology of Gall Insects*; (ed.) T N Ananthakrishnan, (New Delhi: Oxford-IBH Publishing Co.) pp. 1–10
- Davidson E H 1968 *Gene activity in early development*, (New York: Academic Press)
- Gopinathan K 1984 *Studies on the developmental morphology and histochemistry of some galls induced by thrips (Thysanoptera: Insecta) from southern India*, Ph.D. Thesis, University of Madras, Madras
- Johansen D A 1940 *Plant Microtechnique* (New York: McGraw-Hill)
- Mace M E and Howell C R 1974 Histochemistry and identification of condensed tannin precursors in roots of cotton seedlings; *Can. J. Bot.* **52** 2423–2426
- Maresquelle H-J 1980 La morphogenese dans l'impasse? Reflexions d'un cecidologue; *Bull. Soc. Bot. Fr., Actual. Botan.* **127** 9–16
- Raman A and Ananthakrishnan T N 1983 Studies on some thrips (Thysanoptera: Insecta) induced galls. 2. Fine-structure of the nutritive zone; *Proc. Indian Natl. Sci. Acad.* **B49** 525–561
- Raman A and Ananthakrishnan T N 1984 Biology of gall thrips (Thysanoptera: Insecta); In *Biology of Gall Insects*; (ed.) T N Ananthakrishnan (New Delhi: Oxford-IBH Publishing Co.), pp. 107–128
- Rao T A 1957 Comparative morphology and ontogeny of foliar sclereids in seed plants. I. *Memecylon* L; *Phytomorphology* **7** 306–330

Spore shedding in *Aglaothamnion cordatum* (Borgesen) Feldmann. Mazoyer—(Rhodophyta, Ceramiales) of Visakhapatnam coast

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Abstract. Effect of environmental factors on the shedding of tetraspores in *Aglaothamnion cordatum* was studied. Submerged condition of the plants and salinities between 30–40‰ were found to be favourable for the maximum shedding of tetraspores. Light intensity of 2000 lux and 4:20 L:D regime found to be the suitable conditions for maximum liberation of tetraspores. Spore output varied at different times of the day and the highest values were recorded between 10 PM and 2 AM.

Keywords. Spore shedding; *Aglaothamnion cordatum*.

1. Introduction

Studies on the liberation of algal spores in relation to their environmental factors are very few (Yamasaki *et al* 1957; Matsui 1969; White and Boney 1969; Umamaheswara Rao and Kaliaperumal 1983; Umamaheswara Rao and Subba Rangaiah 1980; Subba Rangaiah 1983, 1984a, b; Subba Rangaiah and Umamaheswara Rao 1983; Subba Rangaiah *et al* 1975). The effect of some environmental factors on the shedding of monospores in *Porphyra vietnamensis* Tanaka et Ho was studied by Subba Rangaiah (unpublished results). The literature available on the shedding of spores reveal that all algae do not respond to similar sets of factors, even if they are growing in the same habitat. The author studied the autecology of *Aglaothamnion cordatum*, with a view to understand the biology and ecology and in this paper only the effect of environmental factors on spore shedding is given.

2. Material and methods

Plants of *Gracilaria corticata* on which *Aglaothamnion cordatum* was growing as an epiphyte were collected from August to December 1984 and brought to the laboratory in plastic bags filled with seawater. The tufts of *Aglaothamnion cordatum* were removed from the host (*G. corticata*) and washed thoroughly with sterile seawater. Then they were brushed carefully and again washed to minimize the diatoms present on the surface. The tetrasporic plants were identified under low power microscope.

The material thus selected was used in different experiments. To study the effect of desiccation on tetraspore shedding, the plants were exposed to air, out side the laboratory ($30 \pm 2^\circ\text{C}$; R.H. 78%). At 15 min intervals, the material thus exposed to air was transferred to Petri-dishes containing sterile seawater and brought to the laboratory conditions. Different salinities were prepared to study the effect of salinity on spore shedding following the method given by Subba Rangaiah *et al* (1975),

ranging from 10–50‰ and the spore output was determined at 10, 20, 30, 40 and 50‰ salinities. Light intensity of 1500 lux was provided by cool white fluorescent lamps both for desiccation and salinity experiments.

The effect of light intensity on tetraspore shedding was studied at 0, (dark), 500, 1500, 2000 and 2500 lux. Four different photoperiods were used to study its effect on tetraspore output (viz., 24:0, 0:24, 4:20, 18:6 light: dark regimes). All the above experiments except desiccation experiments were started at 6 PM at ambient temperature ($29 \pm 2^\circ\text{C}$ 76 R.H.) and after 24 hr, the spores liberated in the Petri-dishes were counted following the method given by Umamaheswara Rao and Kaliaperumal (1983). Whereas for desiccation experiments, taking into account the time of exposure to air, only after 24 hr from the starting of each experiment, the spores liberated in the Petri-dishes were counted.

The diurnal periodicity of tetraspore shedding was studied by changing the Petri-dishes every 4 hr. These experiments were also started at 6 PM and the Petri-dishes were changed at 10 PM, 2 AM, 6 AM, 10 AM, 2 PM and 6 PM. The spores liberated in the Petri-dishes were counted to know the time of peak shedding of spores in a day.

In all the above experiments 8–10 replicates were used and after each experiment is over, the weight of the plants used and the average was expressed in spores per gram fresh weight per day.

3. Results

The effect of desiccation on tetraspore shedding is shown in figure 1. The number of spores liberated in the control (0 hr of exposure) was found to be the maximum. There

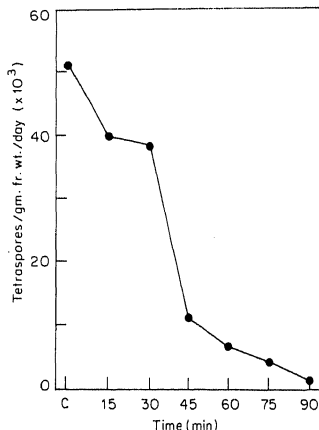


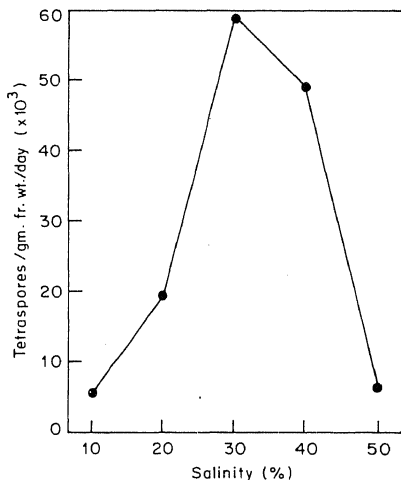
Figure 1. Effect of desiccation on tetraspore shedding in *A. cordatum*.

fall in the number of spores was noticed. After 45 min of exposure onwards, the decrease is slow upto 90 min.

Figure 2 depicts the spore output at different salinities. Peak output of tetraspores at 30‰ salinity and slight decrease at 40‰ salinity was noticed. The number of spores liberated at 10‰ and 50‰ are found to be almost equal.

Light intensity of 2000 lux was found to be more favourable for the maximum liberation of tetraspores in *Aglaothamnion cordatum* (figure 3). The spore output was noticed even in dark condition and sudden increase is seen at a light intensity of 500 lux. From 500–2000 lux, the increase of spore shedding is slow and sudden fall was observed at 2500 lux. Effect of photoperiod on spore shedding is expressed in figure 4, which denotes that maximum spore shedding is seen at a photoperiod of 4 hr and minimum number in complete darkness.

Figure 5 shows the diurnal periodicity of tetraspore shedding in a day. As illustrated in figure 5, the quantities of spores liberated varied at different times of the day. The spore emission was maximum between 10 PM and 2 AM. The liberation of spores started from 6 PM and continued upto 2 PM. Sudden increase in the spore output was seen between 10 PM and 2 AM and there was no liberation of spores between 2 PM and 6 PM. The spores which were liberated at the peak period are found to be more viable and healthy than the spores liberated in the other periods.



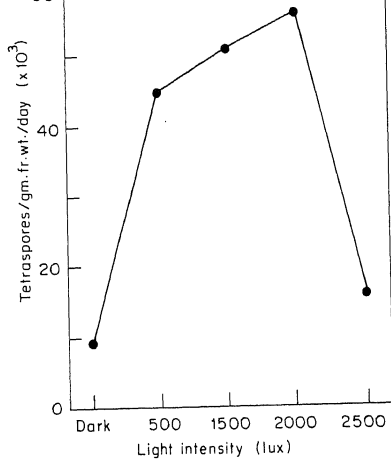


Figure 3. Effect of light intensity on tetraspore liberation in *A. cordatum*.

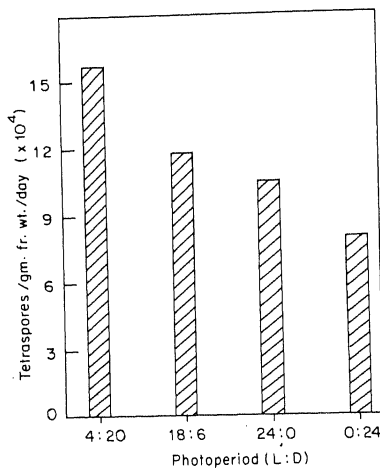


Figure 4. Effect of photoperiod on tetraspore emission in *A. cordatum*.

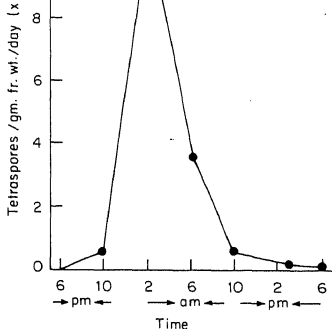


Figure 5. Diurnal periodicity on the shedding of tetraspores in *A. cordatum*.

4. Discussion

As described previously in the members of Gigartinales (Umamaheswara Rao and Subba Rangaiah 1980) and Gelidiales (Umamaheswara Rao and Kaliaperumal 1983) in *A. cordatum* also submerged condition of the plants found to be favourable for the maximum shedding of tetraspores. In *P. vietnamensis* (Subba Rangaiah, unpublished results) desiccation of the thalli upto 3 hr of exposure to air had no adverse effect on the shedding of monospores, which confirms the fact that, intertidal algae are more susceptible than sub-littoral algae. In *Gloiopeltis tenax* and *Gloiopeltis furcata* (Matsui 1969) desiccation of the fronds accelerated spore liberation.

The maximum shedding of tetraspores between 30‰ and 40‰ salinity in the case of *A. cordatum* is almost similar to the condition that was described earlier in *Gracilaria corticata* (Subba Rangaiah *et al* 1975). Significant influence was not noticed at salinities from 17 to 52‰ in the case of *Gloiopeltis tenax* and *Gloiopeltis furcata* (Matsui 1969). In the case of *Hypnea valentiae* (Umamaheswara Rao and Subba Rangaiah 1980) and *Porphyra vietnamensis* (Subba Rangaiah, unpublished results) the maximum output of tetraspores and monospores were found between 20‰ and 30‰ salinities. The present findings agree with the results obtained by Yamasaki *et al* (1957), White and Boney (1969) in the case of *Porphyra tenera* and *Acrochaetium sp.*

In *Gracilaria corticata* complete darkness was found to be congenial for the maximum shedding of tetraspores (Umamaheswara Rao 1976; Umamaheswara Rao and Subba Rangaiah 1980). But in the present study, light intensity of 2000 lux was found to be favourable for the maximum shedding of tetraspores. In *Porphyra*

Monostroma (Ohno 1971; Ohno and Nozawa 1972) higher light intensities and long day conditions enhanced spore or gamete liberation. In *Conchocelis* stage of *Porphyra rosengurtii* also, the monospore release was noticed with the increase of illumination (Kapraun and Luster 1983). In *Porphyra vietnamensis* also (Subba Rangaiah, unpublished results) the maximum monospore shedding from the leafy thalli was noticed at a light intensity of 2500 lux.

The time of peak shedding in a day varies in different algae. In *G. corticata* and *Gracilariopsis sjoestedtii*, the period of peak shedding of spores was noticed between 2 AM and 6 AM (Umamaheswara Rao 1976; Umamaheswara Rao and Subba Rangaiah 1981; Subba Rangaiah 1983, 1984b). The time of peak shedding of tetraspores in a day in the case of *Aglaothamnion cordatum* is similar to the time of carpospore shedding in *Hypnea valentiae* (Umamaheswara Rao and Subba Rangaiah 1981; Subba Rangaiah and Umamaheswara Rao 1983). In *Gracilaria textorii* the maximum output of tetraspores and carpospores was noticed between 2 PM and 6 PM (Umamaheswara Rao and Subba Rangaiah 1981; Subba Rangaiah 1984a).

From the above findings it is clear that, the submerged condition, salinities between 30‰ and 40‰, light intensity of 2000 lux and short day conditions, were found to be favourable for the maximum shedding of tetraspores in *Aglaothamnion cordatum*.

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References

- Kapraun D F and Luster D G 1983 Field and culture studies of *Porphyra rosengurtii* Coll et Cox (Bangiales, Rhodophyta) from North Carolina; *Bot. Mar.* **23** 449–457
- Kurogi M and Sato S 1967 Effect of photoperiod on growth and maturation of *Conchocelis* thallus of *Porphyra umbilicalis* (L.) Kuetz. and *Porphyra pseudocrassa* Yamada et Mikatmi; *Bull. Tohoku Reg. Fish. Res. Lab.* **27** 111–130
- Matsui T 1969 Studies on the liberation and germination of spores in *Gloiopeltis tenax* (Turn.) J. Ag. and *G. furcata* Post et Rupr.; *J. Shimonoseki Univ. Fish.* **17** 185–231
- Ohno M 1971 The periodicity of gamete liberation in *Monostroma*; *Proc. Seventh Int. Natl. Seaweed Symp.* 405–409
- Ohno M and Nozawa K 1972 Observations of spore formation and photosynthetic activities on *Monostroma nitidum*; *Bull. Jpn. Soc. Phycol.* **20** 30–35
- Subba Rangaiah G 1983 Seasonal growth reproduction and spore shedding in *Gracilaria corticata* J. Ag. of the Visakhapatnam Coast; *Proc. Indian Nat. Sci. Acad.* **B49** 711–718
- Subba Rangaiah G 1984a Growth, reproduction and spore shedding in *Gracilaria textorii* (Sur.) J. Ag. of the Visakhapatnam Coast; *Phykos* **23** 242–247
- Subba Rangaiah G 1984b Spore shedding in *Gracilariopsis sjoestedtii* (Kyl.) Dawson (Rhodophyta, Gigartinales) *Proc. Symp. on All India Symposium on Marine Plants, Goa* 59–64
- Subba Rangaiah G and Umamaheswara Rao M 1983 Seasonal growth, reproduction and spore shedding in *Hypnea valentiae* (Turn.) Mont.; *Proc. Indian Acad. Sci. (Plant Sci.)* **92** 373–382
- Subba Rangaiah G, Umamaheswara Rao M and Rao B G S 1975 Effects of salinity on spore shedding in *Gracilaria corticata*; *Curr. Sci.* **14** 717–718
- Umamaheswara Rao M 1976 Spore liberation in *Gracilaria corticata* J. Agardh growing at Mandapam; *J. Exp. Mar. Biol. Ecol.* **21** 91–98

- Umamaheswara Rao M and Subba Rangaiah G 1980 Effects of environmental factors on the shedding of tetraspores of some Gigartinales (Rhodophyta); *Proc. Symp. Coastal Aquaculture* (in press)
- Umamaheswara Rao M and Subba Rangaiah G 1981 Effects of environmental factors on the diurnal periodicity of tetraspores of some Gigartinales. Rhodophyta; *Xth International Seaweed Symp.*, Sweden 209–214
- Umamaheswara Rao M and Kaliaperumal N 1983 Effects of environmental factors on the liberation of spores from some Red algae of Visakhapatnam Coast; *J. Exp. Mar. Biol. Ecol.* **70** 45–53
- White E B and Boney A D 1969 Experiments with some endozoic and endophytic *Acrochaetium* spp.; *J. Exp. Mar. Biol. Ecol.* **3** 246–274
- Yamasaki M, Sitanaka M and Fukuhara A 1957 Studies on the ecology of the Conchocelis phase of *Porphyra tenera* Kjellw. 4 on the influence of diluted seawater on the growth and maturity of Conchocelis; *Bull. Jap. Soc. Sci. Fish.* **23** 195–198

Succinate dehydrogenase and peroxidase activities in leaf epidermis and stem of some angiosperm species growing near a fertilizer complex

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Abstract. The enzymes succinate dehydrogenase and peroxidase were localized histochemically in the leaf epidermal cells and in the young stems of: *Annona squamosa* L., *Coccinia indica* W. and A., *Bougainvillea spectabilis* Willd., *Azadirachta indica* L., *Ficus benghalensis* L., *Lantana camara* L. var. *aculeata*, *Mangifera indica* L., *Manilkara hexandra* (Roxb) Dub., *Streblus asper* Lour., *Syzygium cumini* L., *Tamarindus indica* L., and *Zizyphus oenoplia* L., from the areas around the Gujarat State Fertilizer Company, near Baroda (22°–30° N latitude, 73°–60° E longitude), and nearly-normal area of Vallabh Vidyanagar (22°–50° N latitude, 73°–10° E longitude). The soil types, temperature, rainfall, humidity and agricultural patterns of the two localities are identical. Intensities of enzyme activity vary in different cell types of the epidermis and in various tissues of the stem. Intensities of succinate dehydrogenase and peroxidase in epidermal cells is low in majority of the pollution-affected plants. Except in *Azadirachta indica* and *Manilkara hexandra*, stem of all the other pollution-affected species showed lower succinate dehydrogenase activity than in the normal. Vascular parenchyma in the stems of pollution-affected plants of *Azadirachta indica* and *Manilkara hexandra* showed high succinate dehydrogenase activity than that in the normal, whereas in extravascular regions it is almost identical with that of the normal stem. Peroxidase activity in the stem of pollution-affected plants of *Manilkara hexandra* and *Streblus asper* is higher than that in normal, and in rest of the species it is lower than or almost the same as in the normal.

Keywords. Air pollution stress; epidermis; peroxidase; SDH.

1. Introduction

Peroxidase is a well known enzyme in plant tissue with a wide number of isoenzymes, and its physiological implications are immense. Oxidation of auxins (Galston *et al* 1953; Jensen 1955), involvement in ethylene synthesis (Yang 1967; Mapson and Wardale 1971), lignin metabolism (Siegal 1962; Hall and Sexton 1972; Hepler *et al* 1972), hydroxylation of proline (Ridge and Osborne 1970), disease resistance (Seever *et al* 1971) are some of the important roles ascribed to this enzyme.

Peroxidase activity is used for monitoring and mapping air pollution areas by Keller (1974). Peroxidase activity increases in ozone sensitive varieties of soybean (Curtis *et al* 1976). High peroxidase activity in roots and leaves of *Zea* and leaves of *Medicago*; and low activity in the roots of *Medicago* treated with lead is reported by Maier (1978). Succinate dehydrogenase (SDH) is an essential part of Krebs's cycle and occurs in all the aerobic cells.

Enzyme systems are the most logical places to be expected for the attack of air pollution (Heck *et al* 1973). However, reports are lacking on the histoenzymological variations caused by pollutants, hence the present investigation is an attempt in this direction.

Pollution-affected samples were collected from fields near the Gujarat State Fertilizer Company, Fertilizer Nagar, Baroda. Normal (nearly) samples were collected from the University Botanical Garden, Vallabh Vidyanagar, where the pollutants found in Fertilizer Nagar are not present. The aerial distance between the two localities is 21 km. Fertilizer Nagar is located on 22°–30' N latitude and 73°–60' E longitude; and Vallabh Vidyanagar on 22°–50' N latitude and 73°–10' E longitude. The rain-fall, soil types, macro- and micronutrient levels in the soil, temperature and agricultural patterns are identical in the two locations. The major pollutants, as monitored by us, in the Fertilizer Complex area are ammonia—42.412 (in March) to 291.06 (in September) $\mu\text{g}/\text{m}^3$;

Table 1. SDH-activity in epidermal peelings.

Species	Guard cells		Subsidiary cells		Epidermal cells		Hairs	
	N	P	N	P	N	P	N	P
<i>A. squamosa</i>	3+	2+	3+	2+	3+	+	NA	NA
<i>A. indica</i>	4+	2+	4+	2+	4+	3+	NA	NA
<i>B. spectabilis</i>	4+	2+	3+	2+	2+	2+	3+	+
<i>C. indica</i>	3+	3+	3+	3+	2+	2+	NA	NA
<i>F. benghalensis</i>	3+	2+	3+	2+	3+	2+	3+	2+
<i>L. camera</i>	2+	+	+	—	+	—	+	—
<i>M. indica</i>	3+	2+	3+	2+	3+	2+	NA	NA
<i>M. hexandra</i>	3+	3+	3+	4+	2+	3+	NA	NA
<i>S. cumini</i>	3+	2+	3+	2+	2+	+	NA	NA
<i>T. indica</i>	4+	+	4+	+	3+	+	NA	NA

N, normal; P, pollution-affected; NA, not applicable; -, negative; +, negligible; 2+, mildly positive; 3+, less intensely positive and 4+, intensely positive.

Table 2. SDH-activity in young stem.

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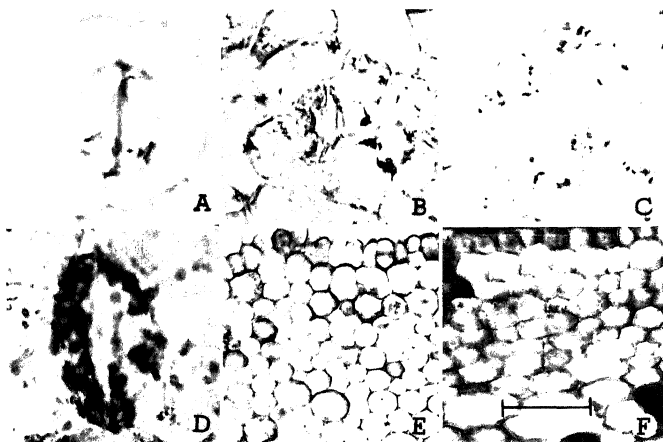
arsenics, carbon monoxide, hydrocarbons, hydrogen fluoride, lead, oxides of nitrogen, ozone—all traces; particulates— $78.4 \mu\text{g}/\text{m}^3$; phosphorus—trace; and sulphur dioxide— 45.016 (in March) to 124.712 (in September) $\mu\text{g}/\text{m}^3$. The level of NO_2 was $47.00 \mu\text{g}/\text{m}^3$ in September. The levels of pollutants in March and September were different due to the change in the wind direction over the fields of experiments.

The enzymes succinate dehydrogenase and peroxidase were localized in the abaxial epidermal peelings of the leaves of 10 species (table 1) and free hand sections of young stems (from the mature plants) in 12 species (table 2), following the methods of Nachlas *et al* (1957) for SDH, and Molner and La Croix (1972) for peroxidase. Controls for enzyme reactions were maintained as recommended in the respective procedure.

3. Observations

3.1 SDH-activity in epidermal peelings

SDH-activity is described as negative, negligible (figure 1A), mildly positive (figure 1B), less intensely positive (figure 1C) and intensely positive (figure 1D), depending on the absence or presence and the intensity of the activity. All the cell types of epidermal structure of normal as well as pollution-affected leaves with exception of subsidiary cells and epidermal cells of pollution-affected leaves of *Lantana camara*, showed positive SDH activity. The intensity of enzyme activity varies in various cell types of the same species, and in the same cell type of the different species. In pollution-affected leaf



Figures 1 A–F. SDH-activity. A–C, E, F. Pollution-affected samples. D. Normal. A. *T. indica*. B, D. *B. spectabilis*. C. *M. hexandra*. E. *S. asper*. F. *S. cumini*. Scale represents 12

granules, whereas in normal leaves it is in the form of large but a few granules. In *Manilkara hexandra*, SDH-activity in subsidiary cells and epidermal cells of pollution-affected leaves is higher than those of the normal ones; whereas the activity in guard cells is almost identical in both the samples. In remaining species pollution-affected leaves showed lower SDH-activity than the normal ones. Intensity of SDH-activity in various cell types is presented in table 1.

3.2 SDH-activity in young stem

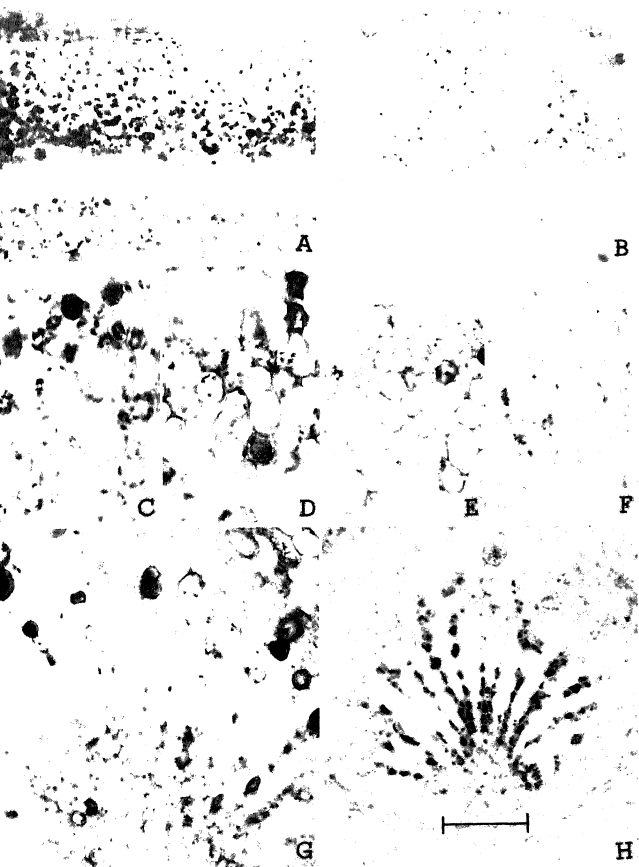
Figures 1E, F, 2C and A represent negligible, mildly positive, less intensely positive and intensely positive, respectively. Intensity of SDH-activity varies in various regions of stem of the same species as well as in the similar tissue types of stem of different species (table 2). In majority of the species, pollution-affected young stem shows low SDH-activity than the normal (*Tamarindus indica* in figures 2A,B). Pollution-affected stems of *Azadirachta indica* and *M. indica* and *M. hexandra* show high SDH intensities in xylem parenchyma than that in the normal stem (figures 2C-H, *M. hexandra*). Pollution-affected and normal stems of *Zizyphus* show more or less identical SDH-activity in all the respective tissue types. Nonetheless, the xylem fibres and pith cells show slightly high SDH-activity in the pollution-affected stem than those of the normal one.

3.3 Peroxidase activity in epidermal peelings

The epidermal cells of most of the species have granular form of peroxidase localization, but in a few others it is amorphous. The hair bases of *Annona squamosa* and all the leaf epidermal cell types of *Bougainvillea spectabilis* show amorphous form. The cells of *S. cumini* showed both the forms of peroxidase localization (figure 3C). Guard cells in figures 3A–D represent negligible, mildly positive, less intensely positive and intensely positive activity, respectively. *B. spectabilis* (figure 3D), *L. camara*, *M. indica*, *M. hexandra*, *S. cumini* and *T. indica* (figure 3A) showed positive activity in cell walls. Intensity of enzyme localization varied in different cell types of the same species as well as in the same cell type of different species (table 3). Except in *M. hexandra*, the pollution-affected leaves of all the species showed lower enzyme intensity than those of the normal condition. In *M. hexandra* the enzyme activity in pollution-affected as well as normal leaves is identical. In *F. benghalensis*, guard cells of normal leaves showed high enzyme activity in the regions adjacent to the walls of the pores (figure 3E), whereas it is low in pollution-affected leaves (figure 3F).

3.4 Peroxidase activity in young stem

Intensity of enzyme in different regions of the stem varies in each species, and it also varies in the same tissue types from species to species (table 4). Figures 3G–J represent negligible, mildly positive, less intensely positive and intensely positive activity, respectively. Majority of the species (both normal and pollution-affected) showed high enzyme activity in cortical and phloem regions as compared with the other regions of the stem. Most of the enzyme activity is centralized in the inter-cellular spaces and cell walls. Intra-cellular enzyme activity in these regions is localized in granular form, and in



Figures 2 A-H. SDH-activity in young stem. A, C, E, G. Normal. B, D, F, H. Pollution-affected. A, B. *T. indica*. C-H. *M. hexandra*. Scale represents 62 micrometers in A-F; 52 micrometers in G, H.



Figures 3A-J. Peroxidase activity. **A, B, F, G, J.** Pollution-affected. **C-E, H, I.** Normal. **A.** *T. indica*. **B.** *M. hexandra*. **C.** *S. cumini*. **D.** *B. spectabilis*. **E, F, I.** *F. benghalensis*. **G, H.** *C. indica*. **J.** *S. asper*. Scale represents 15 micrometers in **A-C, E, H**; 21 micrometers in **D**; and 62 micrometers in **G-J**.

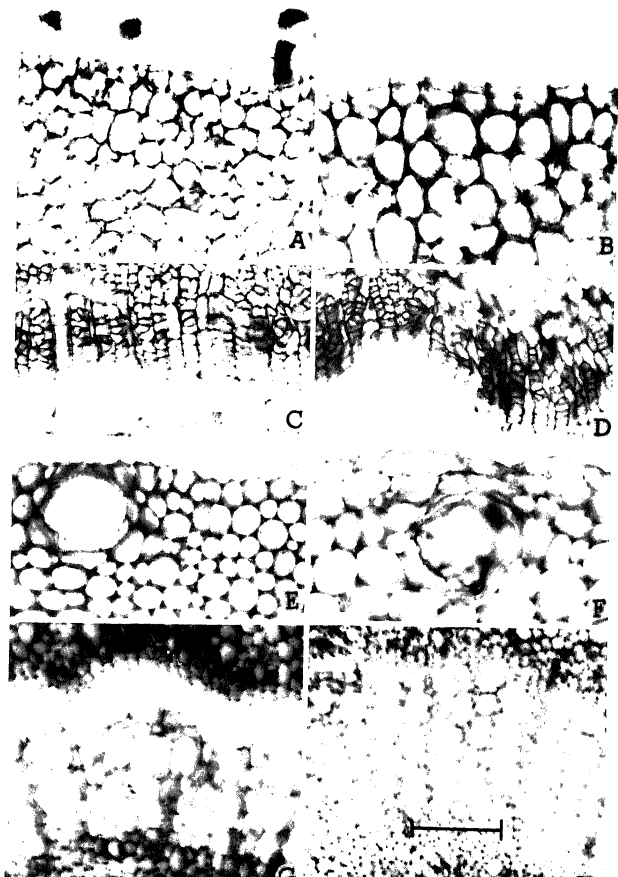
Table 3. Peroxidase activity in epidermal peelings.

Species	Guard cells		Subsidiary cells		Epidermal cells		Hairs		Cell walls	
	N	P	N	P	N	P	N	P	N	P
<i>A. squamosa</i>	3+	2+	3+	3+	2+	+	3+	2+	—	—
<i>A. indica</i>	4+	3+	+	—	—	—	NA	NA	—	—
<i>B. spectabilis</i>	4+	+	+	+	2+	+	3+	+	4+	2+
<i>C. indica</i>	2+	+	2+	+	2+	+	NA	NA	—	—
<i>F. benghalensis</i>	4+	+	+	+	—	—	2+	+	—	—
<i>L. camera</i>	2+	2+	2+	—	3+	+	3+	2+	2+	+
<i>M. indica</i>	2+	+	+	+	+	+	NA	NA	2+	+
<i>N. hexandra</i>	2+	2+	+	+	+	+	NA	NA	+	+
<i>S. cumini</i>	3+	+	3+	+	3+	+	NA	NA	2+	+
<i>T. indica</i>	+	+	+	+	3+	2+	NA	NA	4+	3+

Table 4. Peroxidase activity in young stem.

Species	Epidermis or bark		Cortex				Phloem		Cambium		Protoxylem		Xylem axial		Parenchyma ray			P ₁
	N	P	Outer		Inner		N	P	N	P	N	P	N	P	N	P	N	
<i>A. squamosa</i>	3+	2+	2+	2+	2+	2+	4+	3+	2+	2+	3+	2+	2+	2+	2+	2+	—	
<i>A. indica</i>	4+	3+	4+	3+	3+	2+	3+	2+	3+	2+	4+	3+	2+	+	3+	+	2+	
<i>B. spectabilis</i>	3+	2+	3+	2+	3+	2+	4+	2+	2+	+	3+	2+	2+	+	2+	+	2+	
<i>C. indica</i>	2+	2+	2+	2+	2+	+	+	—	+	—	3+	2+	+	—	+	—	+	
<i>F. benghalensis</i>	—	—	3+	2+	2+	+	3+	+	3+	+	3+	+	2+	+	3+	+	2+	
<i>L. camara</i>	2+	2+	+	+	3+	2+	4+	3+	+	—	4+	3+	3+	2+	3+	+	2+	
<i>M. indica</i>	—	—	3+	+	3+	+	3+	2+	3+	+	3+	+	+	+	+	+	+	
<i>M. hexandra</i>	—	—	2+	3+	2+	3+	3+	4+	3+	4+	—	—	+	+	+	+	2+	
<i>S. asper</i>	—	—	3+	4+	2+	3+	2+	4+	+	3+	2+	4+	3+	3+	3+	2+	2+	
<i>S. cumini</i>	—	—	4+	3+	4+	3+	4+	3+	+	+	3+	4+	+	+	+	+	2+	
<i>T. indica</i>	3+	3+	4+	3+	3+	3+	4+	3+	4+	3+	4+	2+	2+	2+	2+	2+	4+	
<i>Z. oenoplia</i>	3+	3+	3+	3+	3+	3+	3+	3+	3+	2+	4+	3+	—	—	—	—	2+	

N, P, —, +, 2+, 3+, 4+, as in table 1.



respiratory rate, under experimental conditions, in some plants (Mac Dowell 1965; Pell and Brennan 1973; McLaughlin and Barnes 1975). Contrary to this Lee (1967) reported reduced respiration in tobacco treated with ozone. In the present studies we have noticed both the increased and decreased SDH-activity, indicating the respiratory rates. The reduced SDH-activity may be attributed to the detrimental effect of pollution causing elements or compounds on these species. Due to the presence of pollutants in the cells, the metabolic machinery must have been impaired to a larger extent. This is evident from the ultrastructural changes in mitochondria observed in some angiosperm species (Sakunthala Devi 1981). On the other hand, *A. indica* and *M. hexandra* showed high levels of SDH-activities which might be due to the resistance or the adaptability of these species to pollutants. In the species which show high SDH-activity, the plants are helped in the maximum utilization of the available food materials. This is evident from the reduced levels of sugars in pollution-affected plants as compared to that in nearly-normal plants (Sakunthala Devi and Patel 1983). However, other species, which have low SDH-activity, also showed low sugar contents (Sakunthala Devi and Patel 1983). This is directly related to decreased chlorophyll contents. Moreover, in *M. hexandra* reduction in chlorophyll contents is very little as compared to reduction in sugars (Sakunthala Devi and Patel 1983). This suggests that the reduced sugar contents are due to high respiration in cells. Low water potential is reduced by pollutants, which need to be supported from active ascent of sap. The high SDH-activity in cambium and xylem parenchyma may have some role in rapid ascent of sap to compensate the low water potential caused by pollutants. Earlier reports on this aspect, however, are based on the experimental conditions. Whereas, our results are based on prolonged and chronic exposure of vegetation to pollutants. These are combined with the interactions of natural changes in the environments.

Keller (1974) and Curtis *et al* (1976) reported increased peroxidase activity in the plants exposed to fluoride and ozone. We have also observed similar trends in stems of *M. hexandra* and *S. asper*. When the plants are exposed to oxidant pollutants, organic peroxy radicals are produced. These have to be removed from the tissue and it is quite logical to believe that the increased peroxidase may help in this process. However, the occurrence of low peroxidase activity is difficult to be understood. Nevertheless, it may be attributed to the impairment of metabolism.

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- Bhatia D S and Malik C P 1977 Histochemical studies in stomatal apparatus of *Phaseolus mungo* Linn., *Lathyrus sativus* Linn. and *Quantia elator* Mill; *Folia Histochem. Cytochem.* **15** 315-322
- Curtis C R, Howell R K and Kremer D F 1976 Soy bean peroxidases from ozone injury; *Environ. Pollut.* **11** 189-194
- Galston A W, Bonner J and Baker R S 1953 Flavoprotein and peroxidase as components of the IAA-oxidase system of peas; *Arch. Biochem. Biophys.* **42** 456-470
- Hall J L and Sexton R 1972 Cytochemical localization of peroxidase activity in root cells; *Planta* **108** 103-120
- Heck W W, Taylor O C and Heggstad H E 1973 Air pollution research needs: Harbaceous and ornamental plants and agriculturally generated pollutants; *APCA J.* **23** 257-266
- Hepler P K, Rice R M and Terranova W A 1972 Cytochemical localization of peroxidase activity in wound vessel members of *Coleus*; *Can. J. Bot.* **50** 977-983
- Jensen W A 1955 The histochemical localization of peroxidase in roots and its induction by indole acetic acid; *Plant Physiol.* **30** 426-432
- Keller T 1974 The use of peroxidase activity for monitoring and mapping air pollution areas; *Eur. J. For. Pathol.* **4** 11-19
- Lee T T 1967 Inhibition of oxidative phosphorylation and respiration by ozone in tobacco mitochondria; *Plant Physiol.* **42** 691-696
- Mac Dowell F D H 1965 Stages of ozone damage to respiration of tobacco leaves; *Can. J. Bot.* **43** 419-427
- Maier R 1978 Activity and multiple forms of peroxidase in *Zea mays* and *Medicago sativa* treated and non-treated with lead; *Phyton* **19** 83-96
- Mapson L W and Wardale D A 1971 Enzyme involved in the synthesis of ethylene from methionine or its derivatives in tomato; *Phytochemistry* **10** 29-39
- Mc Laughlin Jr S B and Barnes R 1975 Effects of fluoride on photosynthesis and respiration of some south-east American forest trees; *Environ. Pollut.* **8** 91-96
- Molner J M and La Croix L J 1972 Studies of the rooting of cuttings of *Hydrangia macrophylla*: enzyme changes; *Can. J. Bot.* **50** 315-322
- Nachlas M M, Tsow K C, De Souza E, Cheug C S and Seligman A M 1957 Cytochemical demonstration of succinic dehydrogenase by the use of a new-P-Nitrophenyl substituted ditetrazole; *J. Histochem. Cytochem.* **5** 420-436
- Pell E J and Brennan E 1973 Changes in respiration, photosynthesis, adenosine 5'-triphosphate and total adenylate content of ozonated pinto bean foliage as they relate to symptom expression; *Plant Physiol.* **51** 378-381
- Poovalah B W and Rasmussen H P 1974 Localization of dehydrogenase and acid phosphatase in the abscission zone of bean leaves; *Am. J. Bot.* **61** 68-73
- Ridge I and Osborne D J 1970 Hydroxy proline and peroxidase in cell walls of *Pisum sativum*, regulation by ethylene. *J. Expt. Bot.* **21** 853-856
- Sakunthala Devi G 1981 *Phenological and ultrastructural studies in some angiosperm species growing around fertilizer complex*, Ph.D. Thesis, Sardar Patel University, Gujarat
- Sakunthala Devi G and Patel J D 1983 Variations in vegetation growing near a fertilizer complex. II. Chorophyll, sugar and protein contents; *Biol. Bull. India* **5** 229-235
- SeEVERS P M, Daly J M and Catedral F F 1971 The role of peroxidase enzymes in resistance to wheat stem rust disease; *Plant Physiol.* **43** 353-360
- Siegal S M 1962 *The plant cell wall* (London: Pergamon)
- Yung S F 1967 Biosynthesis of ethylene; ethylene formation from methionol by horse-radish peroxidase; *Arch. Biochem. Biophys.* **122** 481-487

Ecological studies in the paper mill effluents and their impact on the river Tungabhadra: Heavy metals and algae

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Abstract. Certain metals have been investigated in paper mills effluent channel and in the river Tungabhadra in relation to their distribution, seasonal fluctuations and their effect, if any, on algae. The concentrations of various metals analysed are in the order of $Zn > Cu > Pb > Ni > Co > Mn$. In general these metals indicated an increase in their concentration along with the increase in the concentrations of chlorides, total hardness, sulphates and total alkalinity. Blue-greens and diatoms seem to be more tolerant to these ions than Chlorophyceae. *Stigeoclonium* exhibited very good growth at high concentrations of zinc, copper and nickel and at low concentrations of cobalt and lead. *Schizomeris* attained good growth when the lead concentration was high and cobalt was low.

Keywords. Heavy metals; algae; paper mill effluents; river Tungabhadra.

1. Introduction

The term 'heavy metal' although not rigidly defined is generally held to refer to those metals having a density > 5 (Passow *et al* 1961; *cf.* Whitton and Say 1975, p. 287). Although some metals, including heavy metals are needed by living organisms for various metabolic processes (Whitton and Say 1975), the physiological and metabolic roles or requirements of such heavy metals as mercury, lead, cadmium and silver are not properly understood. In contrast to herbicides, pesticides and other potential toxicants, which can undergo breakdown, albeit extremely slowly, heavy metals cannot be eliminated from a water body and they persist in sediments from where they may be released slowly into the water. After their release from sediments, heavy metals may again pose serious hazards to aquatic organisms, including algae (Rai *et al* 1981).

Patrick (1978), Whitton (1970, 1980, 1984), Welch (1980) and Stokes (1983) have carried out lot of work on heavy metals in aquatic ecosystems concerned either with toxicity or accumulation, especially for algae. Rai *et al* (1981) have reviewed the relationships between algae and heavy metals.

In nature, the presence of these metals depends on various environmental conditions. The principal sources of heavy metals have been dealt at length by Williams *et al* (1974). Previous studies have shown higher concentrations of these metals associated with the growth of industrialization, though it is difficult to generalise in view of the differences with the type of industry and specific operation, the same product may result in different amounts of metals being contributed to aquatic environment.

The present paper deals with the analysis of effluents from the Rayalaseema paper mills at Kurnool let out in an open channel and their effect after entering the river Tungabhadra. Attention is focussed on heavy metals and their effect on algae inhabiting both in the effluents and in the river.

domestic wastes and effluents in large quantity from Rayalaseema paper mills. Two sampling sites were selected, one in the effluent channel and another after its entry in the river. The effluent channel is an outlet from paper mills factory which runs for about 1½ km through a small village and finally joins the river Tungabhadra. In the channel certain filamentous algae like *Stigeoclonium tenue* and species of *Oscillatoria* were growing luxuriantly throughout the period of investigation. The river station is highly polluted and the colour of the water is dark brown. The bottom is muddy mixed with some silt. The flow of the water is greatly reduced as the river is very wide and somewhat deep.

Surface water samples were collected from both the sampling sites in polythene containers at monthly intervals. The samples were kept in an ice-box and transported to the laboratory. After returning to the laboratory 250 ml of sample was digested by using concentrated nitric and perchloric acids. The digested solution was diluted to 500 ml with redistilled water and then filtered through a porcelain filter crucible into a thoroughly cleaned 100 ml volumetric flask. Finally the solution was made up to 100 ml. Aliquots of this solution were taken for the determination of different metals. For the estimation of lead, ammonium acetate was used to dissolve the lead sulphate (APHA 1971). Final estimation was done with the help of an Atomic Absorption Spectrophotometer (Perkin-Elmer, 2380).

Algae were collected by following the field technique adopted by Blum (1957) and as described in detail by Venkateswarlu (1969). 250 ml of surface water, from both the stations was collected in a wide mouthed bottle of 500 ml capacity. Five uniform sized pebbles (approx. 2" × 2"), colonized by algae were carefully picked up from the habitat with a pair of clean forceps and transferred to the bottle. After returning to the laboratory these pebbles were scraped with a scalpel and brush. The scraped material was preserved in 4% formaldehyde and the final volume of sample was reduced to 100 ml. This material was used for frequency measurements and species identification. For finding out the frequency of different species of algae, the drop method of Pears *et al* (1946) was used. Altogether, 10 slides were prepared and 12 high power fields were counted in each slide. The total number of species occurring in 120 high power fields under the microscope was noted. The percentages and species composition of different groups of algae were calculated. For filamentous algae field observations were considered.

3. Results

The monthly fluctuations in the values of different heavy metals are shown in figure 1.

3.1 Manganese

Manganese has been shown to be required for algal growth. Some algal cells become chlorotic and some lose their capacity to evolve oxygen in its absence. It has an important role in some enzymatic reactions in the Krebs cycle. High concentrations inhibit algal growth. In the river Tungabhadra manganese was not detectable for more

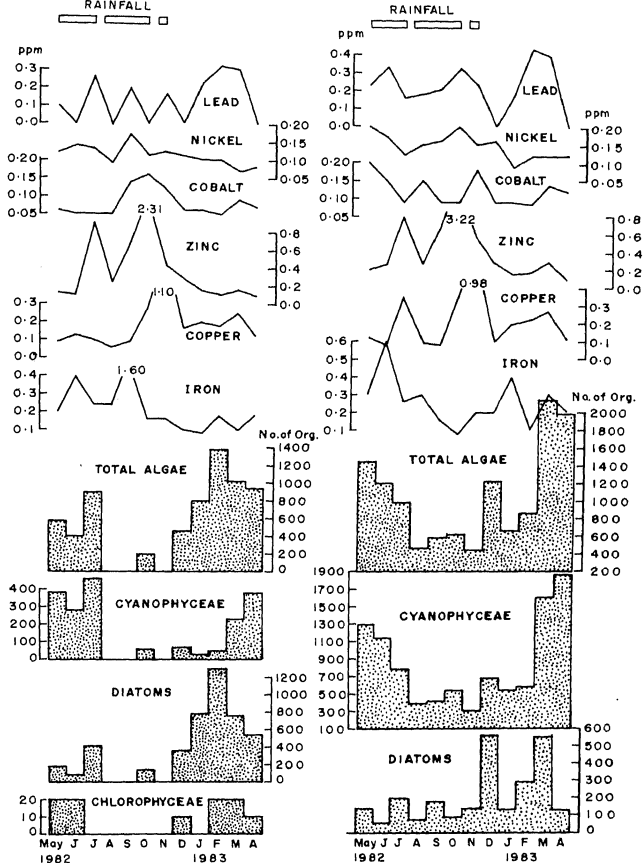


Figure 1. Heavy metals, total algae and different algal groups.

part of the investigation, but it was present in small amounts during the rainy season. This indicates that the contamination was from surface runoff water during floods. In the effluent channel it ranged between 0.03 and 0.08 ppm with an average value of

1981). Copper showed almost the same average concentrations in both the channel and in the river (0.230–0.247 ppm), recording 0.06 and 1.10 ppm as minimum and maximum respectively. Its high values were observed during winter and low values in the rainy season. On the other hand Kimball (1973) and Nammanga and Wilhm (1977) have reported that the concentrations of copper values were low during summer and high in winter.

3.3 Zinc

Zinc is an important micro-nutrient for growth and metabolism of algae and plays a vital role in maintaining the integrity of ribosomes. At high concentrations zinc inhibits the growth of various algae (Whitton 1980).

Zinc concentration in the effluent channel was 0.11–3.22 ppm. In the river water it fluctuated between 0.09 and 2.31 ppm. Its high values were recorded during October 1982 and low values during April 1983 in both the habitats.

3.4 Cobalt

Cobalt concentration fluctuated from 0.03–0.20 ppm and 0.05–0.16 ppm in the effluent channel and river water respectively. Its highest value was attained in May 1982 in the effluent channel and in October 1982 in the river. Its lowest values were observed during February 1983 at both the stations.

3.5 Nickel

The concentration of this metal was high in the effluent channel than in the river. It ranged from 0.07–0.20 ppm. It attained high values during September–October and low values in March in the river water and in January in the effluents.

3.6 Lead

Lead is often considered to be a potential pollutant in drinking water. In the present study lead was recorded almost throughout, except on 2 or 3 occasions. It ranged between 0.16 and 0.44 ppm in the river water and effluent channel respectively. It was high during February and low in May and July. Its concentration was very high in the effluent channel than in the river. In the present study lead concentration was quite high in both the habitats, exceeding the limits prescribed by WHO (0.10 mg/l) and EPA and USPHS (0.05 mg/l) for drinking water.

3.7 Heavy metals and algae

In the present habitats the heavy metal concentrations are in the order of $Zn > Cu > Pb > Ni > Co > Mn$. Blue-greens and diatoms seem to be more tolerant to these ions than Chlorophyceae. Chlorococcales were totally absent in the effluent channel.

concentrations of metals were associated with thick massive growths of *Stigeoclonium* whereas in the river, this alga was observed when the metals concentration specially zinc and nickel were high during October. On the other hand when metals concentration was low, *Cladophora* showed good growth in the river water.

3.8 Heavy metals and benthic algae

Benthic forms serve as good indicator organisms since they develop in an environment where all sorts of substances come in contact with the substratum. Although various metals occur in different concentrations only the resistant species thrive well in the habitat. The benthic algae in the present habitats were more during winter in the river and in summer in the channel. The algae showed varied responses to metals. Nickel, cobalt and copper showed a direct relationship with total algae in the effluent channel, whereas in the river these metals exhibited an inverse relationship. Zinc showed a direct relationship with total algae in both the habitats but lead gave a direct relationship in the river habitat. Iron and total algae were inversely related in both the habitats (figure 1).

3.8.1 Diatoms: An inverse relationship between copper, nickel and cobalt and diatoms was noticed in the river and almost a direct relationship in the effluent channel. A direct relationship between zinc and diatoms was observed in both the habitats. Iron and diatoms were inversely related but lead and diatoms were directly related in the river and inversely in the effluent channel (figure 1). Sometimes direct and sometimes inverse relationships of various metal ions and diatoms could be attributed to the behaviour of the dominant species present in the habitat as only 3 species were found in abundance throughout the period of investigation. *Rhopalodia gibberula* showed a direct relationship with cobalt, copper, nickel and zinc. On the other hand it showed an inverse relationship with lead. *Nitzschia obtusa* var. *scalpelliformis* also exhibited similar relationships in the effluent channel. But *Nitzschia palea* differs somewhat in its tolerance to these metals. With nickel and cobalt an inverse relationship was noticed, whereas with zinc and copper a direct relationship was found (figure 2).

3.8.2 Cyanophyceae: Blue-greens exhibited a direct relationship with zinc and an inverse relationship with iron, copper, cobalt and lead. Nickel fluctuated directly with blue-greens in the effluent channel and inversely in the river (figure 1). In the effluent channel certain species gave interesting relationship with these metals. *Oscillatoria chalybea* and *O. ornata* var. *crassa* showed a direct relationship with copper, cobalt and nickel and an inverse relationship with zinc and lead. *Oscillatoria animalis* and *O. subbrevis* behaved inversely with copper but the latter species showed a direct relationship with zinc and lead (figure 2).

3.8.3 Chlorophyceae: This group was represented by only two filamentous algae i.e. *Stigeoclonium tenue* and *Schizomeris leibleinii* which were present throughout. In the effluent channel these two species were growing well throughout, but their growth was good during different times. The growth of *Stigeoclonium tenue* was considerable during September, October, December and January, whereas the growth of *Schizomeris*

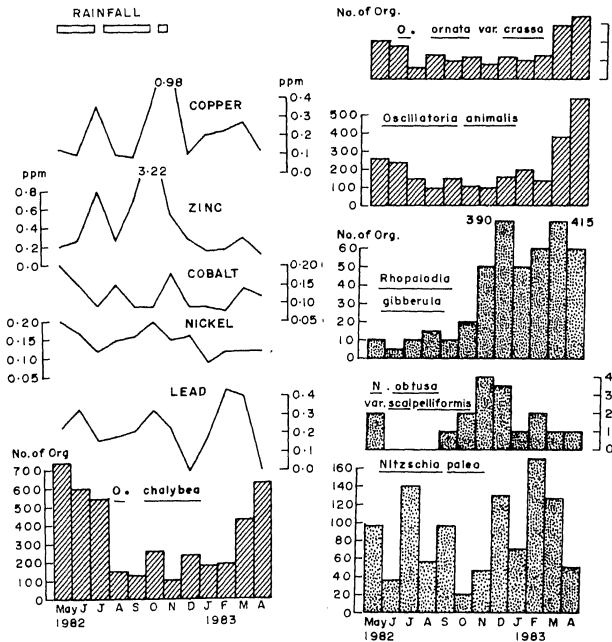


Figure 2. Relationship between heavy metals and certain species of blue-greens and diatoms.

was high during February and March. *Stigeoclonium* gave good response to certain heavy metals. At high concentrations of zinc, copper and nickel and at low concentrations of cobalt and lead its growth was very good. It was also noticed that during October when the zinc concentration was at its maximum the growth of *Stigeoclonium* was in thick masses. On the other hand, *Schizomeris* showed different type of response to metals. This alga attained good growth when the lead concentration was high and cobalt was low.

In the river water this group was recorded in low quantity, hence no definite correlation could be obtained from the data.

4. Discussion

copper and hardness, alkalinity and total dissolved solids. In the present study the alkalinity is due to bicarbonates. The carbonates were either absent or present in very low concentration but did not cause any type of precipitation of heavy metals. This is very well pronounced in the effluent channel where the complete absence of carbonates resulted in the high concentration of these metals as compared to the river water. In the river Tungabhadra apart from dilution, carbonates and somewhat high pH appear to be the factors responsible for the low concentration of these metals. Williams *et al* (1974) reported that calcium carbonate by increasing pH, can remove metals like zinc and copper through adsorption and co-precipitation.

Although algae and heavy metals exhibited some sort of interaction, it may be difficult to attribute that the inter-relationship is exclusively due to these ions. Because of highly polluted nature of habitats the concentration of other pollutants (inorganic and organic) as well plays an important role in the distribution of algae. It is also possible that heavy metals and other pollutants act simultaneously or independently, influencing the flora. Whitton (1984) emphasizes that the influence of metals on algal species and community composition cannot be generalised. Temporal comparisons are rarely possible but spatial comparisons e.g. above and below the point sources of pollution in rivers, have considerable value.

According to Welch (1980) zinc, cadmium, lead, copper, chromium, mercury, silver and nickel are well known toxic heavy metals that can occur in a variety of wastes and cause either acute or chronic effects on organisms. But the toxic effect is generally reduced by several other inorganic and organic compounds which help in the growth and development of algae. Combination of toxicants can display either additive, antagonistic or synergistic effects.

Most of the data on heavy metals and their effects on algae is accumulating specially on diatoms, unicellular and a few filamentous green algae. Several workers have pointed out the tolerance of algal species to certain metals and non-tolerance to others and used them as metal pollution indicator organisms. Patrick (1978) mentioned that algal groups attained dominance at different concentrations of various heavy metals. She reported chromium as toxic to *Scenedesmus* at 5.0 ppm. At lower chromium concentrations diatoms dominated and at higher concentrations the blue-greens dominated. A well illuminated flowing water site with abundant growths of *Stigeoclonium tenue*, but no *Cladophora* at all, should be treated as suspect for metal pollution (Whitton 1970). In the present investigation it is quite evident that in the effluent channel the high concentrations of metals were associated with thick massive growths of *Stigeoclonium* whereas in the river, *Stigeoclonium* was observed when the metals concentration specially zinc and nickel were high during October. On the other hand when the metals concentration was low, *Cladophora* showed good growth in the river water.

5. Conclusions

The paper mill effluents recorded higher concentrations of heavy metals than the river water. In general the metals indicated an increase along with the increase in other ions such as chlorides, total hardness and total alkalinity. There would be some organic and inorganic dissolved compounds in natural waters which act as chelators, bind metal

good indicators of metal pollution. *Rhopalodia gibberula*, *Nitzschia palea*, *Oscillatoria chalybea*, *O. ornata* var. *crassa* and *Stigeoclonium tenue* are important.

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References

- APHA 1971 American Public Health Association. Standard methods for the examination of water and wastewater including bottom sediments and sludges. 13th edn. p. 874
- Blum J L 1957 An ecological study of the algae of the Saline River, Michigan; *Hydrobiologia* **9** 361-400
- Bugenyi F W B 1979 Copper ion distribution in the surface waters of lakes George and Idd Amu; *Hydrobiologia* **64** 9-15
- Kimball K D 1973 Seasonal fluctuations of ionic copper in Knights Pond Massachusetts; *Limnol. Oceanogr.* **18** 169-172
- Namminga H and Wilhm J 1977 Heavy metals in water sediments and chironomids; *J. Water Pollut. Contr. Fed.* **49** 1722-1724
- Passow H, Rothstein A and Clarkson T W 1961 The general pharmacology of heavy metals; *Pharmacol. Rev.* **13** 185-224
- Patrick R 1978 Effects of trace metals in the aquatic ecosystem; *Am. Sci.* **66** 185-191
- Pearsall W H, Gardiner A C and Greenshields F 1946 Freshwater biology and water supply in Britain; *Sci. Publ. Freshwater Biol. Assoc.* **11** 1-90
- Rai L C, Gaur J P and Kumar H D 1981 Phycology and heavy metal pollution; *Biol. Rev.* **56** 99-151
- Stokes P M 1983 Responses of freshwater algae to metals, In *Progress in Phycological Research* (eds) F Round and D J Chapman (Elsevier Science Publ) vol. 2, pp. 87-109
- Venkateswarlu V 1969 An ecological study of the algae of the River Moosi, Hyderabad (India) with special reference to water pollution. 1. Physico-chemical complexes; *Hydrobiologia* **33** 117-143
- Welch E B 1980 *Ecological effects of waste water* (Cambridge: University of Cambridge) p. 337
- Whitton B A 1970 Toxicity of heavy metals to freshwater algae. A review; *Phykos* **9** 116-125
- Whitton B A 1980 Zinc and Plants in rivers and streams In *Zinc in the Environment. Part II. Health Effects* (ed.) J O Nriagu (New York: John Wiley) pp. 363-400
- Whitton B A 1984 Algae as Biomonitoring of heavy metals in freshwaters, In *Algae as Ecological Indicators* (ed.) L E Shubert (London: Academic Press) 257-280
- Whitton B A and Say P J 1975 Heavy metals, In *River Ecology* (ed.) B A Whitton (Oxford: Blackwell Scientific Publ) pp. 286-311
- Williams L S, Aulenbach B D and Clesceri L N 1974 Sources and distribution of trace metals in aquatic environments, In *Aqueous Environmental Chemistry of metals* (ed.) J A Rubin (Michigan: Ann Arbor Sci. Publ., Ann Arbor) pp. 77-127

Seedling anatomy of some Asclepiadaceae

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Abstract. Two-day and eight-day old seedlings of *Asclepias curassavica*, *Calotropis procera* and *Leptadenia pyrotechnica* were studied. The basipetally differentiating epicotylar vasculature and the root-hypocotyl-cotyledonary vasculature are ontogenetically separate units. The cotyledonary node is unilacunar two-trace and the foliar nodes, unilacunar one trace. The unilacunar two trace cotyledonary nodes in advanced families like Apocyanaceae and Asclepiadaceae seem phylogenetically significant.

Keywords. Asclepiadaceae; xylem differentiation; cotyledonary node.

1. Introduction

Vascularisation of the seedling is of particular interest because it is the first representation of the vascular coordination between root and shoot foreshadowed in the procambial system of the embryo. Investigations which help to build an overall picture of this coordination are of great value (Banerji 1961; Kavathekar and Pillai 1977; Pillai and Goyal 1979). This report forms part of an extensive investigation on the seedling anatomy of Bicarpeolatae.

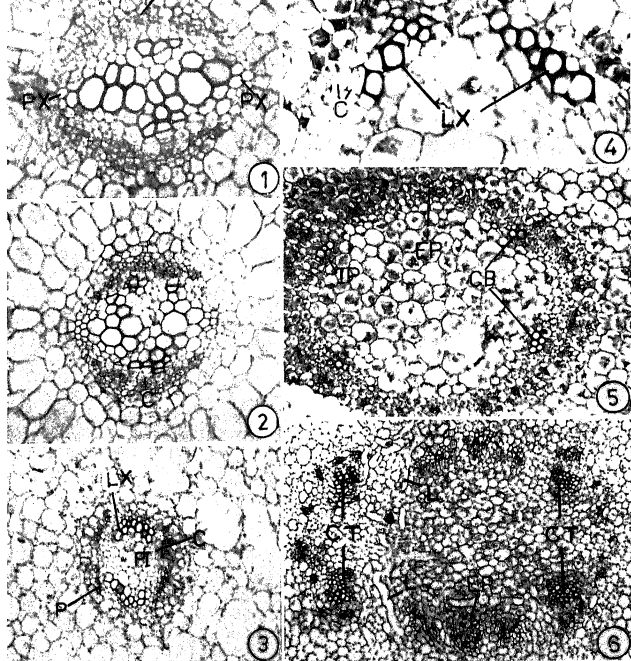
2. Materials and methods

Seedlings of *Asclepias curassavica* L., *Calotropis procera* (Ait.) R.Br. and *Leptadenia pyrotechnica* (Forsk.) Decne were grown in petridishes and pots. Two-day and eight-day old seedlings were fixed in formalin-acetic acid-alcohol (FAA), dehydrated in TBA series and embedded in paraffin. Serial transverse sections cut at 10–15 µm were stained with safranin-light green combination.

3. Results

Germination is epigeal in all the species. The vascular pattern in the 2-day and 8-day old seedlings was basically similar and the measurements presented from eight-day old seedlings represent distances from the radicular end.

The primary root is diarch. In *Asclepias* and *Calotropis* there is a plate of xylem in the centre flanked by the two phloem groups (figure 1). *Leptadenia* shows a small pith. The origin of the pith, marked by the development of a few parenchymatous cells, is observed at 1.06 cm in *Asclepias* and 2.17 cm in *Calotropis* (figure 2). Pith becomes increasingly prominent at higher levels (figure 3). In *Asclepias* the metaxylem appears bifurcated at 1.14 cm level. Fresh xylem differentiating *in situ* from parenchymatous



Figures 1–6. Serial transverse sections of the seedling of *Calotropis* (1–2, 5–6) and *Asclepias* (3–4). 1. The diarch root ($\times 400$). 2. Shows cambium like cells and beginning of pith ($\times 400$). 3. Shows xylem arms, four phloem groups and developing pith ($\times 250$). 4. Shows two xylem arms and differentiation of xylem from cambium like cells ($\times 500$). 5. Shows four collateral bundles and epicotyl procambium ($\times 400$). 6. Shows departure of cotyledonary traces and vascular elements differentiating from epicotyl procambium ($\times 300$).

cells inner to the pericycle add to the xylem arms. Cambium-like cells inner to the phloem cut off some xylem to the inside (figures 2,4). In *Leptadenia* cambium-like cells develop at 2.56 cm level and are less prominent and less active than in the other two species (table 1).

In all the species, lateral expansion of the phloem is followed by the demarcation of each phloem arc into two, making a total of 4 groups. Collateral condition is attained following this and 4 collateral bundles are formed, two flanking each protoxylem pole

Table 1. Seedling measurements.

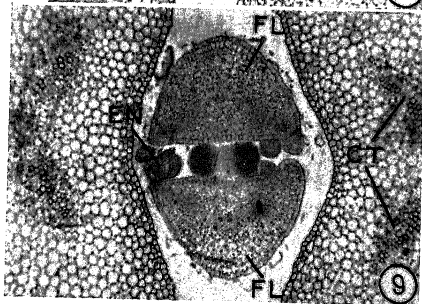
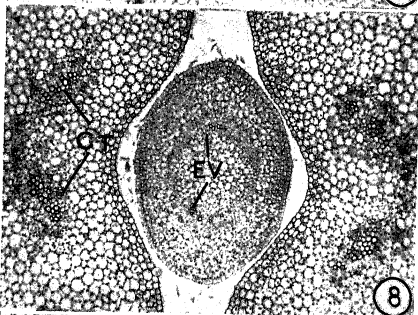
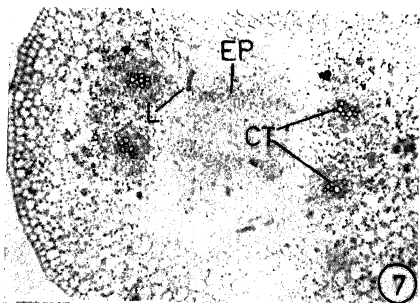
Species	Type of germination	Appearance of laticifers	Beginning of pith	Appearance of cambium-like cells	Formation of collateral bundles	Appearance of internal phloem	Obliteration of protoxylem	Departure of cotyledonary traces
<i>Asclepias</i>	Epigeal	1.0 cm	1.06 cm	1.14 cm	1.15 cm	1.16 cm	1.25 cm	3.12 cm
<i>Calotropis</i>	Epigeal	2.15 cm	2.17 cm	2.15 cm	2.18 cm	2.27 cm	2.20 cm	5.47 cm
<i>Leptadenia</i>	Epigeal	2.34 cm	already present	2.56 cm	2.27 cm	does not develop	2.70 cm	4.01 cm

at 1.15 cm in *Asclepias*, at 2.18 cm in *Calotropis* and 1.27 cm in *Leptadenia* (table 1; figure 5). Pith is well developed at this stage and many laticifers are found associated with the phloem and the cortex. Internal phloem develops at 1.16 cm in *Asclepias* and 2.27 cm in *Calotropis* (figure 5). Two-day old seedlings do not show internal phloem. In *Leptadenia* no internal phloem is observed in the 2- or 8-day old seedlings. In *Calotropis* medullary phloem is present in well-established plants, but absent in the 2- and 8-day old seedlings. Protoxylem is completely obliterated at 1.25 cm in *Asclepias*, 2.20 cm in *Calotropis* and 2.70 cm in *Leptadenia* (table 1).

In *Calotropis* and *Leptadenia*, well-developed arcs of epicotylar procambium with differentiating phloem patches are seen at a level where the cotyledonary traces are not yet organised (figure 5). In *Asclepias* the epicotylar procambium becomes clear only when the cotyledonary traces are in the cortex (figure 7). Near the cotyledonary node, at 3.12 cm in *Asclepias*, at 5.47 cm in *Calotropis* and 4.01 cm in *Leptadenia*, each pair of vascular bundles flanking the original protoxylem poles are observed in the cortex to supply the cotyledons (figures 6, 7). Thus the cotyledonary node in all the species is unilacunar two-trace and the entire root-hypocotyl vasculature supplies the cotyledons. In *Leptadenia* there is a gradual but pronounced reduction in the mass of xylem in the root-hypocotyl vasculature when traced from the root to the cotyledons. The paired vascular bundles in each cotyledon are complete with xylem, inner and outer phloem in *Asclepias* and *Calotropis* and without inner phloem in *Leptadenia*. In *Asclepias* and *Calotropis* the traces remain separate upto about half the length of the cotyledonary lamina giving off lateral branches after which they fuse, whereas in *Leptadenia* the two traces fuse at the cotyledonary base. The arcs or strips of epicotylar procambium form a complete ring of vasculature above the level of attachment of the cotyledons (figure 8). The first (supply at 3.0 cm in *Asclepias* and 5.55 cm in *Calotropis*) pair of leaves are supplied from this ring and the node is unilacunar one-trace.

Branched laticifers are observed at the base of the cotyledonary traces. They are found associated with the phloem, cotyledonary traces as well as in the pith and cortex. In *Asclepias* at the adaxial base of the cotyledons epidermal structures are observed which resemble extrafloral nectaries associated with foliage leaves but they do not show further growth and development in the cotyledons. In *Calotropis* the first pair of leaves show many adaxial extrafloral nectaries (figure 9).

4. Discussion



Figures 7–9. Serial transverse sections of the seedling of *Asclepias* (7) and *Calotropis* (8–9).
7. Shows departure of cotyledonary traces and formation of epicotyl procambium ($\times 250$).
8. Shows cotyledons and the sign of epicotyl.
9. Shows cotyledons and the sign of epicotyl.

that protoxylem elements are discernible *in situ* for varying distances even after an arch, collateral condition is established shows that the interpretation of torsion is not applicable here and seems to agree with Bonnier (1900a, b) in conceiving a gradual upward shifting of the pole of xylem differentiation along the axis.

It has been suggested that in dicots with epigeal germination, the collateral vasculature of the cotyledons is directly continuous with the radial root vasculature and has no direct connection with the epicotyl vasculature. This is supported by Grassley (1932), Esau (1940), Misra Raj (1970), Kavathekar and Pillai (1977) and Pillai and Goyal (1979, 1983). In plants with hypogeal germination the traces for the first leaf primordium seem to be connected with the primary vasculature of the root. Data on *Acacia*, *Albizia* and *Mimosa* with epigeal germination and *Pithecolobium* showing hypogeal germination support this (Sharma 1981; Pillai and Sharma 1983). The species reported upon here show epigeal germination and the root-hypocotyl-cotyledonary and epicotylar vasculatures seem to be ontogenetically separate units. This supports Thoday's (1939) suggestion of double origin of vascular system. Esau (1965) suggests that the discontinuity of xylem initiation in the two vascular systems may be related to the patterned distribution of auxins.

The cotyledonary node is unilacunar two-trace in the species studied. Marsden and Bailey (1955) proposed this as the most primitive nodal condition. The occurrence of unilacunar two-trace cotyledonary nodes in representatives of widely separated families investigated in this laboratory (Goyal 1981 in Cruciferae, Compositae and Pedaliaceae; Kavathekar and Pillai 1977 in Ranunculaceae; Sharma 1981 in Mimosoideae) seems significant.

Bailey (1956) described 8 types of cotyledonary nodes in dicotyledons and a transition from an even number (2) of median traces at the nodes to an odd number (3 or 5) in the lamina. In the species studied, the two traces at the cotyledonary node fuse before entering the lamina in *Leptadenia* (type 'D' of Bailey) and almost half way up the lamina in the other two (type 'C' of Bailey). The data presented also support Bailey's (1956) conclusion that there is no universal correlation between the form and venation of the cotyledons and specific type of nodal structure.

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References

- Bailey I W 1956 Nodal anatomy in retrospect; *J. Arnold Arbor.* 37 269-287
- Banerji M L 1961 On the anatomy of teratological seedlings I. *Cosmos bipinnatus* Cav.; *Proc. Indian Acad. Sci.* B53 10-19
- Bonnier G 1900a Sur l'ordre de formation des éléments du cylindre central dans la racine et la tige; *C. R. Acad. Sci. Paris* 131 781
- Bonnier G 1900b Sur la différenciation de tissu vasculaires de la feuille et de la tige; *C.R. Acad. Sci. Paris* 131 1276
- James A J and MacDaniels L H 1947 *An Introduction to Plant Anatomy* (New York: McGraw Hill Book Co.)

- Esau K 1940 Developmental anatomy of the fleshy storage organ of *Daucus carota*; *Hilgardia* **13** 175–209
- Esau K 1965 *Plant Anatomy* (New York John Wiley & Sons)
- Goyal S C 1981 *Anatomical and morphological studies on some oil-yielding plants*, Ph.D. Thesis, University of Rajasthan, Jaipur
- Govil C M 1973 Seedling and nodal anatomy of *Nycanthus arbortristis* L.; *J. Indian Bot. Soc.* **52** 113–118
- *Grassley F E 1932 *The anatomy of the primary body of Raphanus sativus* M.S. Thesis, Chicago University, Chicago, USA
- Kavathekar K Y and Pillai A 1977 Studies on the developmental anatomy of Ranales. IV. Seedling anatomy of some members of Ranunculaceae; *Phytomorphology* **27** 240–246
- Marsden M P F and Bailey I W 1955 A fourth type of nodal anatomy in dicotyledons, illustrated by *Clerodendron trichotomum*; *J. Arnold Arbor.* **36** 1–51
- Misra Raj D N 1970 *Primary vascular differentiation in certain members of the family Compositae* Ph.D. Thesis, Indore
- Pillai A and Goyal S C 1979 Anatomy of normal and teratological seedlings of *Carthamus tinctorius*; *Phytomorphology* **29** 38–46
- Pillai A and Goyal S C 1983 Developmental anatomy of some oil-yielding plants. IV. Normal and tricotylous seedlings of *Sesamum indicum* L.; *Feddes Repertorium* **94** 87–90
- Pillai A and Sharma K C 1983 Seedling anatomy of some Mimosoideae *Feddes Repertorium* **94** 225–231
- Sharma K C 1981 *Developmental anatomy of some Mimosoideae* Ph.D. Thesis, University of Rajasthan, Jaipur
- Thoday D 1939 The interpretation of plant structure; *Nature (London)* **144** 571–575

* Not seen in original

Contribution to the anatomy of palm fruits—Cocosoid palms

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Abstract. Mature fruit structure of 8 species of Cocosoid palms representing 8 genera distributed in 3 alliances has been described along with developmental account in 2 species. The diagnostic features have been discussed in the light of the work done in other members of this group.

Keywords. Palm fruits; Cocosoid palm; fruit structure.

1. Introduction

Considering the abundance of fossil palm fruits in the tertiary deposits of India and the lack of literature on the anatomy of fruits of living palms, a scheme on anatomical investigation of fruits of different groups of palms has been undertaken by this laboratory and the present contribution deals with the anatomical account of Cocosoid palm fruits.

The fruit anatomy of this group has attracted the attention of early workers like Winton (1901), Wiesner (1873) and Juliano (1926) who have contributed to the fruit structure of *Cocos nucifera*. Guerin (1949), in his exhaustive comparative account, has referred to the fruit structure of many genera of this group. More recently, Murray (1973) briefly traced the endocarp formation in *Arecastrum romanzoffiana*, *Arikuryroba schizophylla* and Robertson (1977) has dealt with the development of fruit and seed in *Jubaeopsis caffra*.

Fruits in Cocosoid palms are typically one seeded fibrous drupes developing from tricarpeal syncarpous gynoecium with trilobular ovary having one ovule in each locule. They are characterised by tough epicarp, spongy fibrous mesocarp and bony endocarp having three characteristic 'pits' or 'eyes' on its surface.

The seed which tightly fits into the endocarp cavity has deep brown tanniniferous seed coat and horny, solid or hollow, homogeneous or less commonly ruminated endosperm.

Based on the type of ovules, the position of eyes on the endocarp and the embryo in the seed, the group is traditionally divided into 3 subgroups or alliances—the *Cocoid* with anatropous ovules, basal eyes and the embryo, the *Bactroid* with hemianatropous ovules, lateral eyes and the embryo and the *Elaeoid* with orthotropous ovules, apical eyes and the embryo.

The mature fruit and seed anatomy of *Cocos nucifera* Linn. *Arecastrum romanzoff-*

2. Material and methods

The materials for the present investigation were mainly procured from Indian Botanical Gardens, Calcutta and Victoria Garden, Bombay.

Customary methods of microtomy were followed. The hard tissues were softened with 10% of HF or KOH prior to dehydration. Sections were stained with aniline-blue and safranin.

The deposition of tannin and lignin in the tissues was ascertained by histochemical tests prescribed by Jenson (1962).

3. Observation

3.1 Mature fruit anatomy

3.1.1 Pericarp: The pericarp is differentiated into tough epicarp, the middle massive fibrous mesocarp, spongy in *Cocos nucifera*, fleshy mucilaginous in other species studied here; and the inner stony endocarp with 'pits' or 'eyes'.

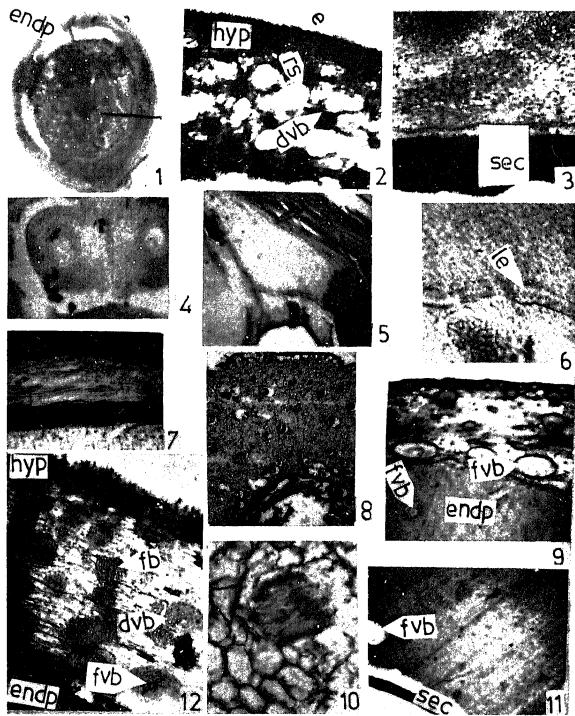
The epicarp consists of outer epidermis and the subjacent hypodermis. The epidermal cells are squarish to rectangular in transectional views with a thick layer of cuticle on the outer tangential walls in all the species except in *B. major* where the cuticle is quite thin. The cells are papillate in *A. romanzoffiana* (figure 30), *A. schizophylla* (figure 32), *A. speciosa*, *S. coronata* (figures 32, 34) and *E. guineensis*. The hypodermis is not sharply defined from underlying mesocarp in *B. major*. In others it consists of squarish to rectangular, compactly arranged somewhat thick walled cells filled with tanniniferous material in *A. speciosa* and *E. guineensis* (figure 36); it is 2-3 layered in *C. nucifera* (figure 33), *A. romanzoffiana* (figure 30), *S. coronata* (figure 34) and *A. caryotifolia* (figure 39). The tissue is interrupted by equidistantly arranged round stegmatiferous fibre strands in all the species except in *A. caryotifolia*.

The mesocarp is bulky and accounts for a major volume of the pericarp in all the species. The ground tissue of the mesocarp contains mechanical, conducting and protective elements. The occurrence, the association and relative distribution of these elements in the mesocarp have often diagnostic value and form basis for topographic divisions of the mesocarp into outer and inner zones as in the majority of Cocoid and Elaeioid species or in outer, middle and inner zones as in *A. speciosa* and *B. major*.

The fibre bundles and the sclerotic strands are the major mechanical elements of the mesocarp. The former are solely restricted to *A. caryotifolia* (figures 38-40) where the outer zone of mesocarp is traversed by a ring of obliquely radially traversing sclerotic strands each with 4-12 angular, stegmatiferous sclereids having large lumina (figure 40). In other species studied, their place is taken over by hypodermal and subhypodermal fibre strands.

Fibrous strands are found in all the species investigated though they are very few in *A. caryotifolia*. They are round to oval in transectional view and have lignified fibres. The peripheral ones are stegmatiferous. In the investigated Cocoid and Elaeioid species they exclusively traverse vertically, while in *B. major* (figure 41) and *A. caryotifolia*

figure 39) some of them traverse obliquely or radially (figure 28). In *C. nucifera*, *A. schizophylla*, *S. coronata* (figure 26) and *E. guineensis* (figure 35), these are evenly distributed along with fibrovascular bundles and raphide sacs in the massive outer part of the mesocarp.



Figures 1-12. 1. *A. schizophylla*, longitudinally cut half of fruit \times ns. 2-7. *Syagrus coronata*. 2. T.S. of the fruit wall in sector \times 125. 3. T.S. of the endocarp and seed coat in sector \times 125. 4. L.S. of the ovary passing through the ovules \times 250. 5. L.S. of the developing seed passing through the micropyle showing stages in differentiation of 'eye' of endocarp \times 125. 6. Part of locular epidermal cells (*le*) and endocarp tissue lying above it \times 1000. 7. T.S. of seed in sector showing two zoned seed coat and cellular endocarp \times 1000. 8-11. *E. guineensis* fruit in T.S. 12. T.S. of the fruit wall in sector \times 125.

In *A. speciosa* (figure 29) and *B. major* (figures 41, 42), the outer narrow mesocarp traversed by 4-6 rows of equidistantly arranged fibre bundles. The inner narrow zone mesocarp abutting on the endocarp is free from fibre strands in all the species except *caryotifolia* and *B. major* where the zone is traversed by radially running strands.

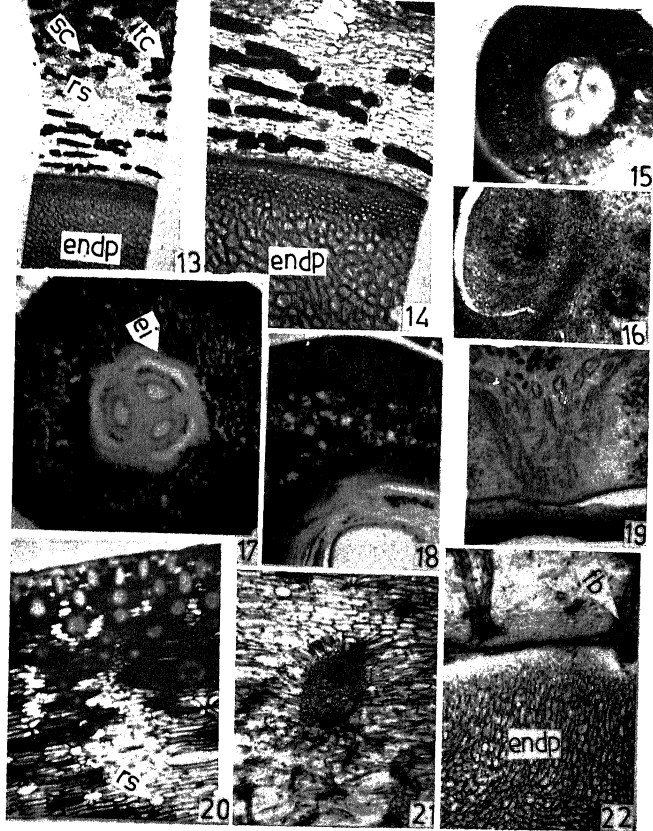
Fibrovascular strands of Cocosoid palms serve both as conducting as well as mechanical tissues. In them the vascular tissue is always associated with stegmatiferous massive sheath of lignified fibres except in *A. caryotifolia* where the fibres are poorly lignified. They traverse the mesocarp vertically in all the species except in *A. caryotifolia* (figure 39) and *B. major* (figures 22, 41), where some run obliquely or radially. The fibrovascular bundles are of concentric and collateral types. The former are oval or circular in transectional view, either hydro- or leptocentric with reduced vascular tissues and massive fibrous sheaths. The collateral vascular bundles are larger than the concentric ones. They have well developed vascular tissue. The fibre sheath is often well developed above and below the vascular tissues.

The concentric vascular bundles are found in all the species but form prominent features of the mesocarp in Cocoid and Elaeioid species and are less frequent in Bactroid species studied. They are found in either massive midmesocarp as in *speciosa* (figure 29) and *B. major* (figure 20), or in massive outer mesocarp as in other Cocosoid palms studied. They are typically lacking from the inner mesocarp in all the species.

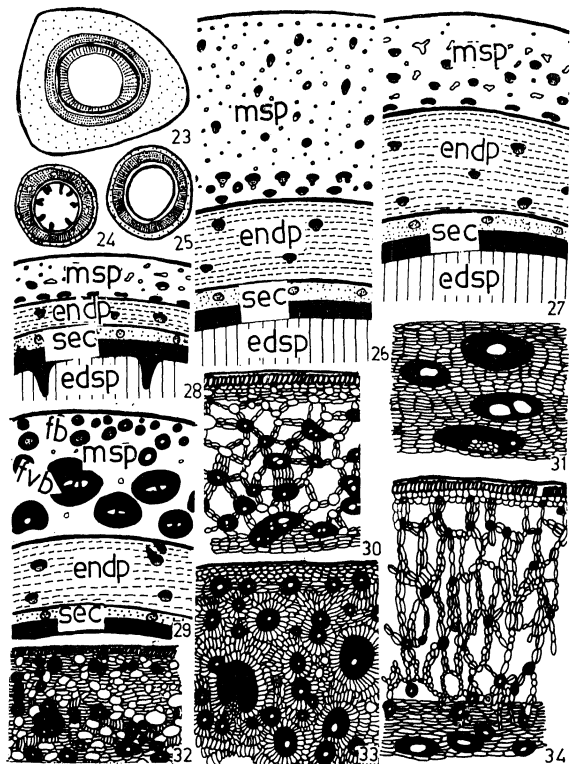
The inner narrow mesocarp of Cocoid and Elaeioid species adjacent to endocarp is characterised by possession of large collateral fibrovascular bundles with massive fibrous sheaths. These are arranged in a single concentric ring in *A. schizophylla* (figure 28) and *A. speciosa* (figure 29); 1-2 rings in *S. coronata* (figure 34) and *E. guineensis* (figures 9, 35); 1-3 rings in *C. nucifera* (figure 26), *A. romanzoffiana* (figures 27, 30). In *nucifera* (figure 26), *A. romanzoffiana* and *S. coronata* (figure 34), the fibrous sheaths are tangentially extended and often marginally fused to form a sclerotic cylinder. In *A. caryotifolia* (figures 19, 39) and *B. major* (figures 22, 41) of Bactroid alliance on the other hand the fibrovascular bundles traverse radially in the inner mesocarp.

In addition to the inner mesocarp, the fibrovascular bundles are also seen distributed along with fibre and concentric vascular bundles and raphide sacs in the massive outer mesocarp in *C. nucifera* (figure 26), *A. romanzoffiana* (figures 27, 30), *A. schizophylla* (figures 28, 32) and *E. guineensis* (figure 35); and in midmesocarp in *A. speciosa* (figure 29), *A. caryotifolia* (figures 38, 39) and *B. major* (figures 20, 41). A few fused fibrovascular bundles are also found in *A. romanzoffiana* (figure 30) and *A. schizophylla* (figure 32). In *S. coronata*, collateral vascular bundles are restricted only to inner mesocarp (figure 34).

The raphide sacs and the tanniniferous cells constitute the protective elements of the mesocarp. Raphide sacs are found in all the species studied. They are thin walled and are distributed along with mechanical and conducting elements. They are typically found in the outer massive mesocarp in Cocoid species and in massive midmesocarp in Bactroid species. In *A. speciosa*, in addition to the midmesocarp where they are numerous, a few are also found in outer thin mesocarp. They are absent in inner mesocarp of all the species studied. They are large, numerous and form conspicuous features of mesocarp in *A. romanzoffiana* (figures 27, 30), *S. coronata* (figures 2, 34), *A. schizophylla* (figures 28, 32), *A. speciosa*, *E. guineensis* (figures 9, 35, 36) and *B. major* (figures 20, 21, 41). They are few, small and do not form prominent features of the mesocarp in *C. nucifera* and *A. caryotifolia*. In all the species they undergo lysis during ripening stages of fruit.



Figures 13–22. 13–19. *A. caryotifolia* fruit T. S. 13. Fruit wall in sector $\times 125$. 14. Part of the same magnified $\times 250$. 15. Ovary after fertilization $\times 250$. 16. Part of the same magnified $\times 1000$. 17. Sector of fruit at free nuclear stage of endosperm showing differentiating endocarp (ei) $\times 70$. 18. The same at advanced stage showing suppression of sterile locules $\times 125$. 19. Inner part of fruit in sector just above 'eye' region of endocarp $\times 125$. 20–22. *Bactris major*, fruit wall in T. S. $\times 125$. 20. Epicarp and mid-mesocarp. 21. Vascular bundle of mid-mesocarp with surrounding ground tissue. 22. Inner part of mesocarp and endocarp.

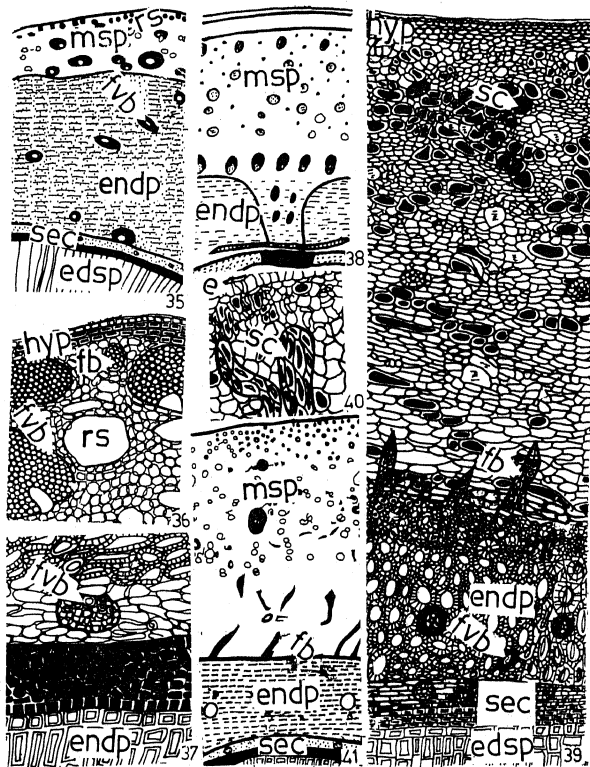


Figures 23-34. Fruits of Cocosoid palms in T. S. 23-25. Gross views. 23. *C. nucifera* $\times 1/3$ ns. 24. *A. schizophylla* \times ns. 25. *S. coronata* \times ns. 26-29. Gross views of fruit sectors. 26. *C. nucifera* $\times 15$. 27. *A. romanzoffiana* $\times 3.5$. 28. *A. schizophylla* $\times 3.5$. 29. *A. speciosa* $\times 2$. 30, 32-34. Mesocarp enlarged $\times 16$. 30. *A. romanzoffiana*. 32. *A. schizophylla*. 33. *C. nucifera*. 34. *S. coronata*. 31. Sector of endocarp in *C. nucifera* $\times 16$.

These spaces are often arranged in a characteristic fashion in *S. coronata* (figures 2, 34).

Tanniniferous or pigmented cells are evenly distributed in outer mesocarp in *A. speciosa* and *B. major* (figures 20, 21). In *A. caryotifolia* (figures 13, 14, 39) they are abundant in midmesocarp forming conspicuous irregular clumps at several places.

pattern throughout the mesocarp in *A. schizophylla* (figures 12, 32), *E. guineensis* (figure 9), *A. caryotifolia* (figures 13, 14, 39) and *B. major* (figures 20, 21); in outer and inner mesocarp in *A. speciosa* and in inner mesocarp in all the species studied. The cells are arranged in a radiating fashion around mechanical and conducting elements in the outer mesocarp of *C. nucifera*, *A. romanzoffiana* (figure 30) and *S. coronata* (figures



2, 34) and in midmesocarp of *A. speciosa*, *A. caryotifolia* (figures 13, 14, 39) and *B. major* (figures 20, 21).

The endocarp is a massive stony zone consisting of highly sclerotic cells in all the species. It is traversed by fibrovascular strands with massive sclerosed, stegmatiferous fibre sheaths. The fibrovascular bundles mostly traverse vertically but in *A. caryotifolia* (figure 14) and *B. major* (figures 22, 41), the peripheral fibrovascular bundles on the outer periphery of the endocarp almost traverse tangentially. The fibrovascular bundles are typically numerous except in *A. caryotifolia* and *B. major*, where they are few and distantly arranged. The bundles are mostly collateral except in *A. speciosa* and *E. guineensis* (figure 35) where some are concentric.

The endocarp at 'eye' sites mainly consists of highly sclerosed, radially stretched cells of locular epidermis with comparatively unsclerosed ground parenchyma cells lying above. A cluster of fibre bundles is seen to traverse radially through the unsclerosed parenchyma.

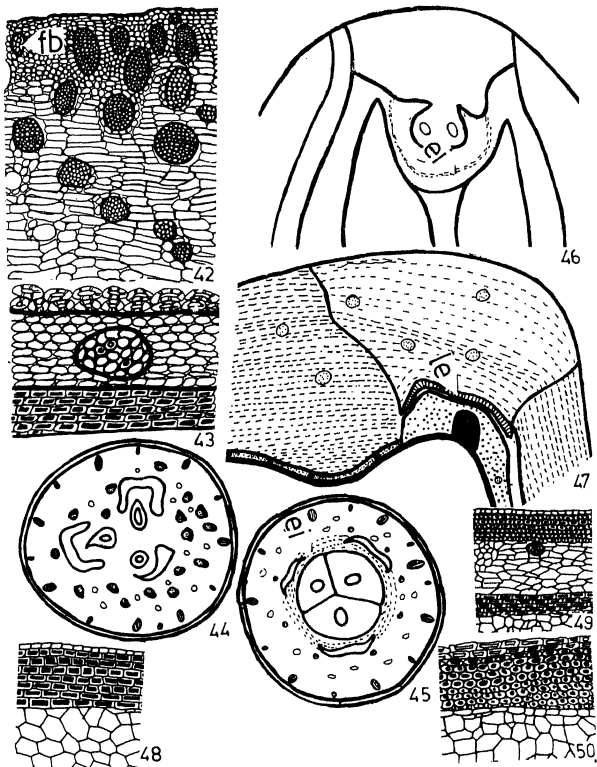
3.1.2 Seed: Seed coat has two zones. The outer massive zone is inseparably fused along the inner surface of the endocarp and contains a ring of vascular strands. The inner delicate zone is non-vascularized and remains adherent to the outer surface of endosperm (figures 26–29, 35, 37, 38, 41 sec).

The ground tissue cells of the outer zone are sclerotic with pitted cell walls (figures 7, 11, 37, 39, 43, 49) and those of the inner zone are filled with tanniferous material (figures 39, 43, 48–50). The endosperm tissue is centrally hollow in *C. nucifera* (figure 23) and *S. coronata* (figure 25). It is completely cellular in other species. The endosperm cells (figures 37, 39) are squarish to rectangular, highly sclerotic with numerous pit-canals in their walls. They are arranged in radial files converging in seed centre. The tissue is regularly ruminated in *A. schizophylla* (figures 24, 28) and rarely so in *S. coronata*. The ruminations appear as radial ingrowths of inner zone of seed coat, produced in close proximity with vascular strands of the outer zone. They are tanniferous and unbranched.

3.2 Developmental anatomy

The 1–3 seeded, mostly one-seeded, fibrous drupe develops from one of the three locules of a tricarpellate syncarpous gynoecium having trilocular ovary with massive septae. A single basal ovule of the locule is anatropous in *S. coronata* (figure 4), orthotropous in *E. guineensis* and hemianatropous in *A. caryotifolia*.

3.2.1 Fruit wall: At anthesis stage, the ovary wall is delimited by an outer epidermis and the inner locular epidermis enclosing ground parenchyma containing provascular strands and raphide sacs. The provascular strands are numerous and evenly distributed in *S. coronata* and *E. guineensis* with progressively increasing size from periphery towards centre. They are few, large and scattered far apart in *A. caryotifolia*. The inner strands surrounding the locules are larger than others in all the 3 species. The differentiation of fibrous sheath is already apparent in many large provascular strands in *E. guineensis* (figure 8). The ground parenchyma has polygonal cells. Many have



Figures 42–50. 42, 43. *B. major*, fruit wall in T. S. $\times 100$. 42. Epicarp and outer part of mesocarp. 43. Seed coat in sector. 44–47. *E. guineensis*. 44. Ovary T. S. $\times 25$. 46. Fertilized ovary showing differentiating endocarp (*el*) $\times 25$. 47. Micropyle and above lying endocarp of developing fruit showing early stage in development of 'eye' $\times 25$. 48. *A. romanzoffiana*, inner seed coat and endosperm $\times 133$. 49, 50. *S. coronata*. 49. Outer and inner seed coat $\times 66$. 50. Inner seed coat and endosperm $\times 133$.

papillate. At early cellular phase of endosperm their radial and outer tangential walls get cutinised. Epidermis of mature fruits have highly papillate cells in *S. coronata* and

E. guineensis and *S. coronata* it is restricted to 1–3 layers of cells situated between the epidermis and the outermost ring of provascular strands. The hypodermal tissue remains 1–3 layered throughout the developmental stages of fruits. Its cells develop tannin granules in *E. guineensis*. In mature fruits the cells are tangentially stretched and rectangular. They are tanniniferous in *E. guineensis*.

The provascular strands of the ovary wall differentiate into fibre, fibrovascular and concentric vascular bundles of the fruit wall. In *S. coronata* and *E. guineensis*, the outermost ring of provascular strands differentiates solely into fibre strands. The inner differentiate into concentric vascular bundles and the innermost ones into fibrovascular bundles with massive fibrous sheaths. In concentric vascular bundles the vascular tissue is represented by phloem while in fibrovascular bundles both xylem and phloem tissues are well developed. The fibres are first to differentiate in provascular strands. These are apparent at early free nuclear phase of endosperm, though in *E. guineensis* differentiation of fibres can be seen in larger provascular strands at anthesis stage itself. The fibres however, remain unligified until cellular stage of endosperm. The xylem and phloem differentiation can be seen by late free nuclear phase of endosperm. The lignification of vessel elements commences at cellular phase of endosperm.

The subhypodermal sclerotic patches in *A. caryotifolia* originate as clusters of enlarged cells in a just fertilized ovary. During early post fertilization developments, they undergo radial elongation and develop silica granules. Finally they differentiate as patches of fibre sclereids with prominent silica crystals associated along their periphery (figure 40).

The oval raphide sacs in 3–4 rows in the ovary wall. They increase in size during post fertilization stages (figures 2, 34) and form conspicuous features of the mesocarp in *S. coronata* and *E. guineensis*. They undergo lysis during ripening stages of fruits along with surrounding parenchyma to form enlarged mucilaginous spaces (figure 34).

Pigmented cells predominate in the ground tissue of the mesocarp during developmental stages in *S. coronata* and *A. caryotifolia* (figures 15, 17, 18). While in the former they are sparsely distributed in the mature mesocarp, in the latter they form irregular clumps in the midmesocarp.

The ground parenchyma cells retain division activity during nuclear phase of endosperm, but are enlarged, and vacuolated at cellular stage. They are loosely arranged in mature mesocarp.

In a fertilized ovary, the tissue destined to develop as endocarp is a cylinder of polygonal meristematic cells towards inner side of the ovary wall immediately surrounding the locules (figure 46 ei). The hyaline nature of this zone gives it a contrasting appearance specially in *S. coronata* and *A. caryotifolia* where rest of the ground tissue dominates in pigmented cells. The cylinder increases in width during early stages of fruit development due to rapid cell divisions. At young free nuclear stage, it is about 20 layered with a number of provascular strands. These are numerous and evenly distributed in *S. coronata* and *E. guineensis*. In *A. caryotifolia* there is a ring of diagonally disposed provascular strands towards periphery with a few additional ones in the ground tissue. The cylinder attains a maximum width which exceeds that of the mesocarp at late free nuclear stage. At this stage its cells are prominently vacuolated but differentiation of vascular tissue in provascular strands is not seen. During further stages of fruit development, the provascular strands differentiate into massive fibrous sheaths. Vascular tissues get differentiated at a slightly advanced stage. During fruit

above the locular epidermis however, remains permanently unsclerosed. The locular epidermal cells elongate radially (figure 6) and get lignified to develop in 'eyes' or 'pits' on the endocarp enclosing the micropyles of the ovules (figure 47).

Lignification of the endocarp cells and the fibres of the pericarp proceeds basipetally during the ontogeny of the fruits. As a result, even in mature fruit these tissues are comparatively less lignified in the basal part than the upper.

3.2.2 Seed: All the three ovules develop upto mature embryosac stage. The outer integument is massive with polygonal cells and the inner is delicate with rectangular cells, both being free only in the micropylar region. The inner epidermis of inner integument organizes the endothelium. The ovular bodies are laterally adnated with septal tissues to various extents in different species (figures 4, 15, 16, 14, 45). The adnation is pronounced in *E. guineensis* where locular cavity lined by locular epidermis is seen only around the micropyles of the ovules (figure 44). In *S. coronata* and *A. caryotifolia* this condition is attained during early post fertilization stages when the volume of the embryosac and the ovules increases rapidly. During these stages, in majority of fruits only one of the three ovule develops faster supressing the others. However, the locular epidermis situated against the micropyles of supressed ovules also shows pronounced radial elongation of its cells followed by lignification to form sterile eyes of the endocarp.

Throughout seed development, the outer integument remains massive. Its polygonal cells undergo frequent radial divisions and infrequent tangential divisions during free nuclear phase of endosperm. As a result, the integument increases in circumference rapidly. Numerous vascular strands are scattered in it. During cellular phase of endosperm, the cells enlarge in size, get slightly thick walled but remain polygonal. The cells of the inner integument are tubular and undergo frequent radial divisions. The division rate is more pronounced in the endothelial cells. Tannin material appears as granules in the cells of inner integument. During cellular phase of endosperm, these granules condense to form dense tannin deposits filling the cell lumen. The seed coat remains adnate to the inner surface of the developing endocarp throughout the developmental stages of seed except at micropylar portions where eyes are organized and the integuments are free from the endocarp. The inner integumentary cells lining the micropylar canal proliferate to form tannin filled plug just after fertilization. The outer integumentary cells in this region divide actively to form a pad of tissue which fits against the developing eye of the endocarp.

The endosperm development is of the free nuclear type. The developing seeds increase in volume mostly during the free nuclear phase of the endosperm. The wall formation progresses centripetally.

While in *E. guineensis* and *A. caryotifolia* the entire seed cavity becomes cellular, in *S. coronata* liquid endosperm remains in centre even at maturity of the seed. The thickening of endosperm cell walls proceeds centrifugally after the tissue becomes fully cellular.

4. Discussion

The mesocarp in the Cocosoid palms examined here as well as in *Juboaepsis caffra* (Robertson 1977) and other members studied by Guerin (1949) is strengthened by a large number of vertically traversing fibre and fibrovascular strands except in *A. caryotifolia* and *B. major*, where radially and obliquely traversing fibrous strands are also met-with. Radial fibre strands have also been recorded in the mesocarp of species of *Astrocaryum*, another Bactroid genus by Guerin (1949).

The raphide sacs are prominently seen in the mesocarp of all the species studied though in *C. nucifera* they are quite few. As the fruits ripen these are converted into vertically enlarged lysigenous mucilaginous spaces. Tanniniferous idioblasts are frequently found in the mesocarp but are predominant in *A. caryotifolia*.

The endocarp which consists of highly sclerotic cells includes some fibrovascular strands also in the species examined here. In *J. caffra* however, Robertson (1977) states that the endocarp is free from vascular strands.

The outer seed coat containing a ring of vascular strands is almost inseparably fused with the inner surface of the endocarp in all the species of the Cocosoid palms studied so far. Robertson (1977) has therefore reconsidered the outer integument to be a part of the endocarp in *J. caffra*.

The Cocosoid palm fruits are characterised by the presence of 3 'eyes' on the endocarp. Basal, apical or lateral position of eyes has been used to divide the group into 3 subgroups by various taxonomists (Drude 1889; Moore 1973). The endocarp at 'eye' sites in the species examined, is restricted to sclerosed palisaded locular epidermal cells into which the micropylar plug formed by inner and outer integuments fits in as noted in *J. caffra* (Robertson 1977).

The ruminations have been observed only in *A. schizophylla*, though occasionally ruminated seeds may develop in *S. coronata* also (Kulkarni 1966). The seeds of *Rhyticocos amara* have also been described to be regularly ruminated (Mc Curran 1960).

The developmental account of fruits in *S. coronata*, *E. guineensis* and *A. caryotifolia* closely parallels that described for *Cocos nucifera* (Juliano 1926) and *J. caffra* (Robertson 1977).

The endocarp at anthesis stage is represented by a narrow zone of hyaline meristematic cells immediately surrounding the locules as reported in *C. nucifera* by Juliano (1926). This zone increases in girth as the fruit develops and comes to have some provascular strands also. As the tissue matures, the cells enlarge variously and get sclerosed. The provascular strands differentiate into fibrovascular strands with lignified fibres. Murray (1973), however, suggests that the endocarp in *A. schizophylla* and *A. romanzoffianum* develops due to sclerification of ground tissue cells along with fibrous sheaths of fibrovascular bundles of the inner part of the fruit wall situated above the locular epidermis.

The locular epidermis solely contributes to the endocarp at 'eye' sites where it comes to have highly radially stretched palisaded cells immediately lying over the micropylar operculum of all the 3 ovules. In this sense as suggested by Robertson (1977) all the three locules take part in the development of the endocarp though only one locule is fertile.

The differentiation of various tissues of the fruit wall takes place basipetally as reported in coconut (Juliano 1926) and *J. caffra* (1977).

post fertilization developments. In *E. guineensis*, a major part of the ovular body appears to be fused with the septal tissues even at mature embryo sac stage as reported in *E. oleifera* by Uhl and Moore (1971). In *S. coronata* and *B. major*, however, major part of the ovular body is free from the ovary wall at mature embryo sac stage, a situation that exists in *C. nucifera* also (Juliano 1926).

As a result of this adnation, right from early post fertilization stages, the integuments of the ovules remain adnated to the inner surface of the developing endocarp excepting at micropylar regions where they remain free from locular epidermis and organize the micropylar plug.

The endosperm development as in other palms (Kulkarni and Mahabale 1974; Mahabale and Biradar 1968; Rao 1959; Shirke and Mahabale 1972) is of free nuclear type and the wall formation progresses centripetally.

References

- Drude O 1889 'Palmae' in Englar and Prantl; *Die naturlichen pflanzenfamilien* Leipzig, ed. 1. Teil 2, abt. 3 1-93
- Guerin H P 1949 Contribution a l'etude du fruit et de la graine des palmiers; *Ann. Sci. Nat. Bot. Serie* 11 10 26-69
- Jenson W A 1962 *Botanical histochemistry* (London: Fruman and Co.)
- Juliano J B 1926 Origin, development and nature of the stony layer of the coconut (*Cocos nucifera* L.); *Philippine J. Sci.* 30 187-200
- Kulkarni A R 1966 *Studies on palms-genus Cocos L in India*; Ph. D. thesis, University of Poona, Poona
- Kulkarni K M and Mahabale T S 1974 Studies on palms; Embryology of *Livistona chinensis* R.Br; *Proc. Indian Acad. Sci.* 67 77-96
- Mahabale T S and Biradar N V 1968 Studies on palms; Embryology of *Phoenix sylvestris* Roxb.; *Proc. Indian Acad. Sci.* B67 77-96
- Mc Currach J C 1960 *Palms of the world* (New York: Harper Bros.)
- Moore H E 1973 The major groups of palms and their distribution; *Gentes Herbarum* 11 27-141
- Murray S G 1973 The formation of endocarp in palm fruits; *Principes* 11 91-103
- Rao C V 1959 Contribution to the embryology of palmae: Part II. Ceroxylineae; *J. Indian Bot. Soc.* 37 47-75
- Robertson F L 1977 Morphology and development of the fruit and seed of *Jubaeopsis caffra* Becc; *Principes* 21 23-29
- Shirke N S and Mahabale T S 1972 Studies on palms: Embryology of *Caryota urens* L.; *Adv. Plant Morphol.* 218-232
- Uhl N W and Moore H E 1971 The palm gynoecium; *Am. J. Bot.* 58 945-992
- Wiesner 1873 *Die rohstoffe des pflanzen* - Leipzig Reiches: p. 436, 789
- Winton A L 1901 Anatomy of the fruit of *Cocos nucifera*; *Am. J. Sci.* 2 265-280

(List of abbreviations in figures: Diminutive vascular bundle, dvb; epidermis, e; endosperm, edsp; endocarp initials, ei; endocarp, endp; fibre bundle, fb; fibrovascular bundle, fvb; hypodermis, hyp; locular epidermis, le; mesocarp, msp; raphide sac, rs; sclerenchyma patch, sc; seed coat, sec; tannin cell/sac, tc).



Stomatal response of chlorocholine chloride and indole-3-acetic acid in *Commelina communis* L.

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Abstract. The apertures of stomata on isolated epidermal peels from both the leaf surfaces of *Commelina communis* were reduced in response to chlorocholine chloride as the concentrations increased from 0.01–10.0 mol m⁻³. When chlorocholine chloride was applied in combination with different concentrations of indoleacetic acid, stomatal closure occurred only when a high concentration of chlorocholine chloride was applied with a low concentration of indoleacetic acid, while low concentrations of chlorocholine chloride did not affect the stimulation of aperture caused by indoleacetic acid treatments. Chlorocholine chloride treatments caused depletion of K⁺ from the guard cells while indoleacetic acid resulted in accumulation of K⁺.

Keywords. Stomatal response; chlorocholine chloride; indoleacetic acid; *Commelina communis* L.

1. Introduction

Control of stomatal behaviour by application of exogenous chemicals which induce their partial closure, in order to check excessive transpirational rates and improve water economy without causing any damaging side effects on the metabolism of plants, would be of great practical importance. It could, for example, be used on field crops to overcome temporary water deficits. A large number of compounds have been screened and tested on different plant species but they are unsatisfactory because they have some side effects or their effects are short lived (Mansfield and Davies 1981). Chlorocholine chloride (ccc) and its allied compounds have been found to increase tolerance to salinity (El Domaty *et al* 1964) and to improve drought resistance (Halevy and Kessler 1963). The present experiments have been performed to investigate the impact of ccc on the opening and closing mechanisms of stomata on isolated epidermal peels from leaves of *Commelina communis*, and its interactions with indoleacetic acid (IAA).

2. Materials and methods

Plants of *C. communis* L. grown in a greenhouse of the Department of Biological Sciences, Lancaster University, Lancaster were used and the techniques for studying stomatal opening and closing were similar to those described by Pemadasa (1981). The plants were first transferred to a growth chamber maintained at 25°C ± 1°C at 95% RH and provided with 140–150 µmol m⁻² s⁻¹ photon flux density, 2–3 days prior to experimentation, so as to acclimatise them to experimental conditions. Two young fully expanded leaves from plants 5–6 weeks old were removed and floated on distilled water with their dorsal surfaces facing upward. From these leaves 5 mm wide strips were cut

peels were then floated over distilled water with their cuticular surfaces facing upward. These epidermal strips were cut into pieces 10×5 mm in size, and 3 pieces were selected randomly for each treatment. The stomatal apertures of 10 stomata taken at random from each epidermal piece were measured after the incubation period, with a Watson Hilux 70 microscope fitted with a projection eye piece.

The epidermal pieces were incubated in 10 ml solution in 5 mm diameter petridishes containing 10 mol m^{-3} morpholino ethane sulphonic acid (MES) buffer at pH 6.15 together with 50 mol m^{-3} KCl, supplemented with CCC and IAA separately or in their various combinations as stated in table 1. The petridishes were placed in a glass water bath at a temperature of $25^\circ\text{C} \pm 1^\circ\text{C}$ and illuminated from below by fluorescent tubes which provided white light of $140\text{--}150 \mu\text{mol m}^{-2} \text{ s}^{-2}$ photon flux density. CO_2 free air was bubbled into the medium at $100 \text{ cm}^3 \text{ m}^{-1}$ through hypodermic needles. The duration of incubation was 3 hr and the experiments were performed between 9 AM and 1 PM (GMT).

Histochemical localization of K^+ in the guard cells was achieved using the techniques of Willmer and Mansfield (1970) and the amount of K^+ was assessed subjectively by arbitrary scoring. Six main categories were recognised (0–5) depending upon the amount of black precipitate of cobalt sulphide present in the guard cells (Pemadasa 1979a,b) and the results were evaluated statistically.

3. Results

Two types of experiments were performed to investigate the mechanism of opening and closing of stomata of *C. communis* in response to the CCC treatments. The responses of stomata to adaxial and abaxial surfaces were studied with different concentrations of CCC (0.01, 0.1, 1 and 10 mol m^{-3}) on an optimal incubation medium (10 mol m^{-3} MES + 50 mol m^{-3} KCl at 6.15 pH) in light and CO_2 -free air. The stomata of both the surfaces responded to CCC by closing, the magnitude of response increasing with the CCC concentration from 0.01– 10 mol m^{-3} . On the ventral surface the stomata were,

Table 1. Showing the presence of K^+ in guard cells as revealed by black precipitate.

CCC mol m^{-3}	IAA mol m^{-3}			
	–IAA	0.01 IAA	0.1 IAA	1 IAA
–CCC	2	<2	2	>3
0.01	2	<2	2	3
0.1	<2	<2	<2	3
1.0	<1	<2	<2	<2
10	0	<1	<1	<1

(0), Absent; (1), black grains present; (2), 1/4 guard cells covered with black precipitate; (3), 1/2 guard cells covered with black precipitate; (4), 3/4 guard cells covered with black precipitate; (5), whole guard cell covered with black precipitate.

partially open in this concentration (figure 1).

In the second set of experiments the incubation medium was supplemented with CCC (0.1, 1 and 10 mol m^{-3}) and different regimes of IAA (0.01, 0.1 and 1 mol m^{-3}). The experiments were performed under similar conditions of light, temperature and CO_2 -free air as in those described previously. It was observed that IAA alone in the absence of CCC removed the differential opening of stomata of adaxial and abaxial surfaces (figures 2 and 3). The apertures of stomata on both the surfaces was almost the same, and this effect was most pronounced in IAA 1 mol m^{-3} , while in the other two concentrations it was less evident. With CCC and varying concentrations of IAA it was observed that in the highest concentration of CCC (10 mol m^{-3}) and the lowest concentration of IAA (0.01 mol m^{-3}) the stomata closed on both the surfaces, but higher concentrations of IAA (1 and 0.1 mol m^{-3}) and lower concentrations of CCC (1, 0.1 mol m^{-3}) produced no significant change in the apertures obtained (figures 2 and 3).

To study the effect of CCC on K^+ influx and efflux in the guard cells, the histochemical localization of K^+ was carried out in the epidermal peels incubated with different concentrations of CCC and IAA, alone or in combinations. It was observed that CCC (10 mol m^{-3}) reduced the influx of K^+ from the incubating medium, as there were only a few dark grains of cobalt sulphide dispersed in the guard cells while more K^+ was observed in the epidermal cells. On the other hand in a medium without CCC, and in a medium with IAA alone, large amounts of K^+ were found the arbitrary scores being 2 and 3 respectively (table 1).

4. Discussion

Plant cell membranes are greatly influenced by the application of exogenous growth substances. Scalla and Gauvrit (1983) proposed that compounds with herbicidal properties can interact with plant membranes either by binding themselves on to the membranous site, thus altering the permeability or fluidity of the membrane, or by

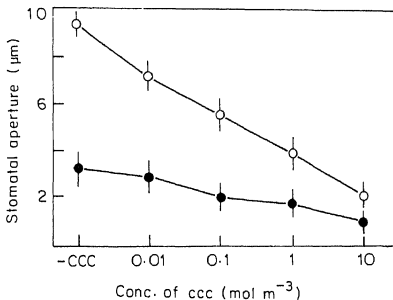


Figure 1. Mean stomatal aperture of adaxial and abaxial surfaces in response to CCC concentrations (○, adaxial; ●, abaxial). Vertical bars represent SD.

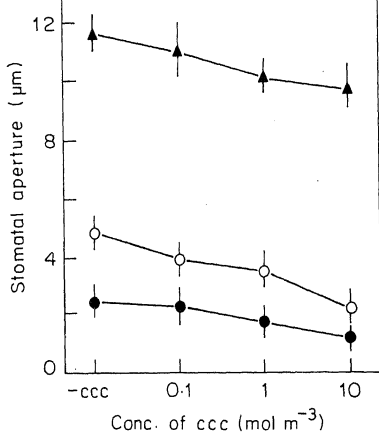


Figure 2. Mean stomatal aperture of adaxial surface in response to different regime of ccc and IAA (○, ●, ▲ represent 0.01, 0.1 and 1 mol m⁻³ IAA). Vertical bars represent SD.

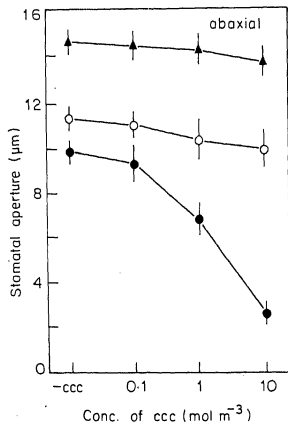


Figure 3. Mean stomatal aperture of abaxial surface in response to different regime of ccc and IAA, (○, ●, ▲ represent 0.01, 0.1 and 1 mol m⁻³ IAA). Vertical bars represent SD.

Scania (1983) reported that the herbicide ianach stimulates K^+ uptake in *Acer* cells. The role of K^+ influx and efflux in changing the solute potential of guard cells is well established (Raschke 1975). Influx of K^+ depends on the membrane potential generated by proton extrusion and the permeability of the membrane to K^+ , while efflux may occur along the concentration gradient (Travis and Mansfield 1979a,b). Thus a net gain of K^+ by the guard cells results in stomatal opening and a net loss causes closure. The present experiments reveal that ccc results in the closure of stomata of both adaxial and abaxial surfaces and the extent of closure depends upon the concentration of ccc. ccc also negates the effect of IAA, which stimulates stomatal opening on adaxial epidermis, removing or reducing the difference in opening between the adaxial and abaxial surfaces. A high concentration of ccc (10 mol m^{-3}) caused a considerable reduction in stomatal aperture even in the presence of IAA (0.01, 0.1 and 1 mol m^{-3}).

Histochemical localization of K^+ in the guard cells gave results that correspond with the stomatal apertures recorded. In ccc-treated epidermal peels, the K^+ accumulation was less in the guard cells and more in the epidermal cells, but in IAA-treated peels more K^+ accumulated in guard cells than in epidermal cells. The combination of a high concentration ccc and a low concentration of IAA resulted in the closure of stomata, but in a low concentration of ccc and a high concentration of IAA the stomata remained open though their apertures were reduced. Thus it appears that ccc either binds itself on to the membrane sites or causes a change in permeability or fluidity of the membrane, resulting in the inhibition of influx of K^+ , or an accelerated efflux of K^+ , from the guard cells. Its antagonistic effect with IAA reveals that it may be either binding itself on to the site of IAA action on the membrane acting competitively with IAA.

Another important known property of ccc is that it increases the salt tolerance (El Damaty *et al* 1964) and drought resistance (Halevy and Kessler 1963; Larter *et al* 1965) in plants. Singh *et al* (1973) observed that after the application of ccc, there is a retardation in growth of the wheat plant, but there is no change in water potential of the leaves when the plants were subjected to water stress; simultaneously, however, there is a high accumulation of proteins in the leaves. Although there is no record of the effect of ccc on guard cell membranes or on the proline content of these cells, it appears from the present experiments that ccc either acts directly on guard cell membranes or it acts through the synthesis of proline or similar compounds like betaine which may affect the membrane leading to reduced K^+ in guard cells, and causing stomatal closure. Finally, it may be suggested that the external application of ccc could to some extent help in overcoming a period of drought, but trials are needed before undertaking such practice on a larger scale.

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- Blein J P and Scalla R 1983 Action of herbicide lenacil on K^+ permeability of *Acer* cells: in *International Symposium - Membranes and compartmentation in Regulation of Plant Functions* Toulouse, France (Abstract)
- El Damaty A H, Kuhn A M and Linser M 1964 A Preliminary investigation on increasing salt tolerance of plants by application of (2-chloro-ethyl)-trimethyl ammonium chloride; *Agrochimica* **8** 129-138
- Halevy A H and Kessler B 1963 Increased tolerance of bean plants to soil drought by means of growth-retarding substances; *Nature (London)* **197** 310-311
- Larier E N, Sanhh M and Sosulski F W 1965 The morphological and physiological effects of CCC on barley; *Can. J. Plant Sci.* **45** 419-427
- Mansfield T A and Davies W J 1981 in *the Physiology of Biochemistry of Drought Resistance in Plants* (eds L C Paleg and D Aspinall). (San Francisco Academic) 314-346
- Pemadasa M A 1979a Movements of abaxial and adaxial stomata; *New Phytol.* **82** 69-80
- Pemadasa M A 1979b Stomatal responses to two herbicidal auxins; *J. Exp. Bot.* **30** 267-274
- Pemadasa M A 1981 Abaxial and adaxial stomatal behaviour and responses to fusicoccin on isolated epidermis of *Commelina communis*; *New Phytol.* **89** 373-384
- Raschke K 1975 Stomatal action; *Ann. Rev. Plant Physiol.* **26** 309-340
- Scalla R S and Gauvrit C 1983 Action of Herbicides on Plant Cell Membrane; in *International Symposium on Membranes and Compartmentation in Regulation of Plant Function*. Toulouse, France (Abstract).
- Singh T N, Aspinall D and Paleg L G 1973 Stress metabolism IV The influence of proline accumulation on wheat (2-chloroethyl) trimethyl ammonium chloride (CCC) & GA_3 on growth and plants during water stress; *Aust. J. Bot. Sci.* **26** 77-86
- Travis A J and Mansfield T A 1979a Stomatal responses of light and CO_2 are dependent on KCl concentration; *Plant Cell Environ.* **2** 319-323
- Travis A T and Mansfield T A 1979b Reversal of CO_2 responses of stomata by fusicoccin; *New Phytol.* **83** 607-614
- Willmer C M and Mansfield T A 1970 Further observations on cation stimulated stomatal opening in isolated epidermis; *New Phytol.* **69** 639-645

A new antheridiogen from the fern *Pityrogramma calomelanos* (L.) Link

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Abstract. A new antheridiogen from *Pityrogramma calomelanos* of the family Polypodiaceae (Sensu Bower) has been extracted and tested on *Pityrogramma calomelanos*, *Onychium siliculosum* (Desv) C. chr. and *Onychium japonicum* (Thbg) Kze. The antheridiogen, here designated as Apit, did not produce uniform effect on the species tested. It promotes dark germination in all, but induces antheridia only in *Pityrogramma calomelanos* and *Onychium siliculosum* under light and dark conditions. The antheridium-inducing capacity of Apit is markedly different on the two ferns, being more vigorous under light condition in *Pityrogramma calomelanos* and less in *Onychium siliculosum*. Contrary to this, more antheridium-bearing prothalli have been counted in the latter under darkness. Higher dilutions are less effective in both. Dark germination has been found most effective in case of *Onychium japonicum*. The results indicate that the intensity of the effect of antheridiogen varies under different conditions and between species of the same genus. Also, an antheridiogen is not specific in the induction of antheridia or dark germination of spores, but it may initiate any of the two or both.

Keywords. Antheridiogen; Apit; Apt.

1. Introduction

Since the discovery of antheridium-inducing substance in *Pteridium aquilinum* by Döpp (1950) several leptosporangiate ferns have been reported to contain such substances which are capable of inducing antheridia (Näf 1956, 1959, 1960, 1965, 1969; Pringle 1961; Näf *et al* 1969; Schedlbauer and Klekowski 1972). Of the several known antheridiogens one from *P. aquilinum* (abbr. Apt) has been extensively investigated and its high degree of specificity has been claimed by Näf *et al* (1975) on the basis of its activity remaining restricted within some polypodiaceous ferns (Sensu Bower) only. The degree of specificity would be sharper when the antheridiogen from the same species collected from two different sources shows different activity (Schedlbauer 1974).

The new antheridiogen hereafter called Apit, which constitutes the subject matter of the present communication has been proposed on the ground of its differential sensitivity as mentioned above and its ability to induce germination under dark condition.

2. Materials and methods

The spores of *P. calomelanos* and *Onychium siliculosum* were collected in October 1978 from the Pokhra Valley, Nepal and those of *O. japonicum* were gathered from the

vicinity of Royal Botanical Gardens, Kathmandu. After surface sterilization with 3% sodium hypochlorite solution the spores of *P. calomelanos* were inoculated in petriplates containing Parker's macronutrients and Thompson's micronutrient culture medium (Klekowski 1969) solidified with 1% agar and autoclaved at 15 lbs pressure for 15 min. After 27 days the entire population of the cordate prothalli having antheridia were removed carefully with forceps and the solidified medium allowed to stay upside down in the same petriplate. The process is known as inverted plate method (Voeller 1966). These plates were then inoculated afresh with surface sterilized spores of *P. calomelanos* to find out whether *P. calomelanos* produced any antheridiogen capable of inducing antheridia in the new gametophytes earlier than control.

After ascertaining that *P. calomelanos* was able to produce antheridiogen, its spores were inoculated in 50 ml flasks containing the solidified growth medium. On the 26th day, 10 ml of sterile double distilled water was added to each of the flasks and placed in the room temperature for 6 hr and then incubated in a freezer for 18 hr. After thawing, the liquid was filtered off and the filtrate diluted 10,000 times (1:10,000) (Endo *et al* 1972). Serial dilutions were prepared by the liquid nutrient medium, which were later autoclaved and after cooling inoculated with the surface sterilized spores of *P. calomelanos*, *O. siliculosum* and *O. japonicum*. One set of each was placed under continuous fluorescent light of 300 ft-c intensity and the others kept in darkness at $24 \pm 2^\circ\text{C}$ in a culture room. After germination the dark set was daily exposed to light for 1 hr and observations recorded on every odd day.

3. Results

3.1 Effect of Apit on germination

3.1.1 *In light*: Two sets of different dilutions of Apit-mixed medium with the spores of *P. calomelanos*, *O. siliculosum* and *O. japonicum* were made and one set of each was placed under 300 ft-c intensity of fluorescent light, whereas the other incubated in dark. The above fern spores do not normally germinate in dark. It was observed on the third day that in light the action of antheridiogen was maximum on *O. siliculosum* where the germination percentage goes on increasing with the increase in dilution (table 1). Under such conditions increasing trend in germination was also observed for *P. calomelanos*, but it was found to be less effective when compared with *O. siliculosum*. The germination at 1:1 dilution was 50% in *O. siliculosum* and 23% in *P. calomelanos*; and 91% in the former and 70% in the latter at 1:10,000 dilution. In case of *O. japonicum* Apit activity was found to be somewhat different, where germination increase was recorded upto 1:100 dilution (76%) but higher dilutions decreased germination and it remains only 39% in 1:10,000 dilution.

3.1.2 *In dark*. When the spores are inoculated in antheridiogen-mixed medium, dark germination was induced. It occurs only in lower dilutions of antheridiogen and higher ones were not effective in case of *P. calomelanos* and *O. siliculosum*. The antheridiogen-induced dark germination was most effective in *O. japonicum* where dark germination was induced in higher dilutions also, though the percentage of germinated spores in

Table 1. Apit-induced germination in light and darkness (%) of spores of *P. calomelanos*, *O. siliculosum* and *O. japonicum* on the 6th day.

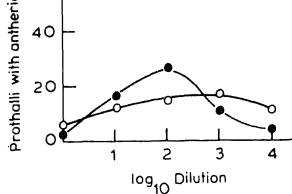
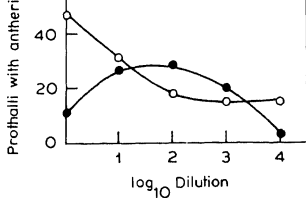
Test	Apit dilution	Germination in 300 ft.c light (%)	95% confidence limit	Germination in dark (%)	95% confidence limit
<i>P. calomelanos</i>	0	52*	41.8-62.1	0*	0-0
	1:1	23	17.4-29.5	7	2.9-13.9
	1:10	34	27.5-41.0	4	1.1-9.9
	1:100	43	32.2-53.2	1	0.0-5.5
	1:1000	57	46.6-66.9	—	0-0
	1:10000	70	63.1-76.3	—	0-0
<i>O. siliculosum</i>	0	43	33.2-53.3	0	0-0
	1:1	50	44-56	19	13-27
	1:10	65	60-70	33	26-42
	1:100	77	72-82	7	2.9-13.9
	1:1000	84	81-87	0	0-0
	1:10000	91	85-95	0	0-0
<i>O. japonicum</i>	0	36	26.7-46.2	0	0-0
	1:1	42	37-49	23	17.4-29.5
	1:10	61	53.9-69.2	39	35-44
	1:100	76	66.9-82.0	40	30-51
	1:1000	66	57.4-73.9	13	9-20
	1:10000	39	35-42	9	6-12

For each observation 100-200 spores were examined.

* Actual germination percentage.

3.2 Antheridium initiation by Apit

Antheridium formation takes about 32 days after inoculation of the spores of *P. calomelanos*; 30 days in *O. siliculosum* and 40 days in *O. japonicum* in control. Spores inoculations with different dilutions of Apit, mixed in growth medium, were observed after 9 days of germination. It is found that the antheridiogen has no effect whatsoever on *O. japonicum*. But in *P. calomelanos* and *O. siliculosum* some interesting results were obtained. Apit starts its activity as early as on the 9th day of germination in *P. calomelanos* when antheridium formation was recorded in lower dilutions of 1:1 and 1:10 (figure 3) but higher dilutions were not effective. The highest dilution tested (1:10,000) initiates antheridium only on the 15th day. Maximum activity of antheridiogen was observed on the 17th day in 1:1 dilution (figure 1) which gradually decreased with increase in dilution. Apit is also effective in inducing antheridia in *O. siliculosum*, but specificity of the antheridiogen is a little different from what was observed for *P. calomelanos* (table 2). In this case the most effective dilutions were 1:10 and 1:100. In 1:1 dilution prothalli with antheridium were observed on the 11th day while no antheridium bearing prothalli could be detected on the 9th day. Higher dilutions were less effective and on the 17th day only a few antheridium bearing



Figures 1 and 2. 1. Per cent prothalli with antheridium in the presence of Apit (\log_{10} dilution) in light. 2. Per cent prothalli with antheridium in the presence of Apit (\log_{10} dilution) in dark; (○) *P. calomelanos*; (●) *O. siliculosus*.

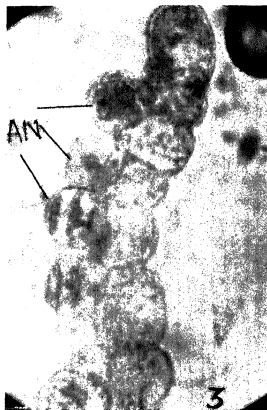


Figure 3. Initiation of antheridium at 1:1 concentration of Apit. AN, Antheridium

3.3 Effect of Apit in light and dark in the initiation of antheridia

The above results obtained under light condition in both the plants are plotted in figure 1 which indicates that the activity of Apit was maximum (c. 47%) in 1:1 dilution on the 17th day, and gradually decreased in higher dilutions. In *O. siliculosus* however, it first increases and then goes on decreasing with the increase of dilution.

Under dark condition Apit is more effective in *O. siliculosus* although the antheridium

Apit dilution	<i>P. calomelanos</i>					<i>O. siliculosum</i>				
	Days after germination					Days after germination				
	9	11	13	15	17	9	11	13	15	17
1:1	5:00	11:00	17:50	21:50	47:18	—	—	4:50	10:00	11:50
1:10	4:50	8:60	15:45	20:00	32:00	—	5:28	12:75	15:40	27:25
1:100	—	4:25	6:80	13:40	19:00	—	4:60	10:25	11:50	29:00
1:1000	—	—	2:20	5:25	16:00	—	—	9:00	9:75	21:45
1:10000	—	—	—	5:15	10:50	—	—	—	—	3:00

bearing prothalli were less than what they were found in light in both the species. *P. calomelanos* was less sensitive under dark in the presence of Apit. The maximum activity that was observed in darkness was 1:100 and 1:1000 when 17 and 26.5% antheridium bearing prothalli were found to be present in *P. calomelanos* and *O. siliculosum*, respectively, and this was followed by the usual decline in the effectiveness. The decline is rapid in case of *O. siliculosum* and slow in *P. calomelanos* (figure 2).

4. Discussion

The present study provides information about Apit, a new antheridiogen extracted from *P. calomelanos*. The activity of Apit has been measured both at the intergeneric and interspecific levels. It is interesting to note that the activity of Apit is distinct from other antheridiogens so far reported by others (Näf 1979). Apit initiated dark germination in spores of all the three ferns tested. Earlier, it had been observed that antheridiogen which induced antheridium promoted dark germination of the spores of the same species (Näf 1966; Weinberg and Voeller 1969; Voeller 1971) with the exception of *Ceratopteris* whose antheridiogen could not induce dark germination in the same (Schedlbauer 1976). Similar activity of GA_3 was in *Anemia phyllitidis* where it promoted dark germination besides inducing antheridia (Schraudolf 1962), and this was later confirmed by Endo *et al* (1972).

The identical functioning of GA_3 and antheridiogen from *A. phyllitidis* has led to the establishment of structural similarity between the two (Nakanishi *et al* 1971). No report is available so far that antheridiogens can cancel light requirement for spore germination in others than its own.

The induction of dark germination by Apit in *P. calomelanos* was expected, but the same in *O. siliculosum* and *O. japonicum* led to the assumption of the presence of two distinct factors, one for dark germination of spores and other for inducing antheridium in Apit. The two factors are probably species-selective because the antheridiogen induced dark germination in all the three species tested but its antheridium-producing capacity was limited only to *P. calomelanos* and *O. siliculosum*, and was not extended to *O. japonicum*. It may be that the factor inducing dark germination is more active than the factor which induces antheridia. One thing, however, seems certain that the antheridiogen which induces dark germination need not necessarily induce antheridia

both possessing the property of antheridial initiation and dark germination. The difference is ascribable to the absence of intergeneric and interfamilial activity of the antheridiogens (Schdlbouer 1974). Like Apt (antheridiogen from *Pteridium aquilinum*) Apit is also found to be active within the alliance of Bower's Polypodiaceae. Yet, it can be clearly distinguished from other antheridiogens in that it is not only able to initiate antheridium but also initiate dark germination in other ferns of the family. The dilution of antheridiogen is another factor which controls germination as well as antheridium formation. Under light, germination percentage goes on increasing with the enhancement of dilution in *P. calomelanos* and *O. siliculosum* but the increase in germination in *O. japonicum* is limited at a certain dilution level after which inhibition started. The answer to why unlike *P. calomelanos* and *O. siliculosum*, the germination percentage in *O. japonicum* decreased with higher dilutions of Apit in light could unfortunately, not be provided at this stage. Raghavan (1976, 1977) proposed that GA₃ activated mRNA of dry spores which is responsible for the synthesis of proteins necessary for germination. The same factor may have played the role in dark germination of spores induced by Apit. The final conclusion can be drawn that Apit is species-selective for induction of antheridia and promotion of dark germination.

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References

- Döpp W 1950 Eine die Antheridienbildung bei Farnen fördernde Substanz in den Prothallien von *Pteridium aquilinum* L. Kuhn; *Ber. dtsch. Bot. Ges.* **63** 139–147
- Endo M, Nakanishi K, Näf U, Mekeon W and Walker R 1972 Isolation of the antheridiogen of *Anemia phyllitidis*; *Physiol. Plant* **26** 183–185
- Klekowski E J Jr 1969 Reproductive biology of the Pteridophyta. II. Theoretical considerations; *Bot. J. Linn. Soc.* **62** 347–359
- Näf U 1956 The demonstration of a factor concerned with the initiation of antheridia in polypodiaceous ferns; *Growth* **20** 91–105
- Näf U 1959 Control of antheridium formation in the fern species *Anemia phyllitidis*; *Nature (London)* **184** 798–800
- Näf U 1960 On the control of antheridium formation in the fern species *Lygodium japonicum*; *Soc. Exp. Biol. Med.* **105** 82–86
- Näf U 1965 On antheridial metabolism in the fern species *Onoclea sensibilis* L.; *Plant Physiol.* **40** 888–891
- Näf U 1966 On dark-germination and antheridium formation in *Anemia phyllitidis*; *Physiol. Plant* **18** 1079–1088
- Näf U 1969 On the control of antheridium formation in ferns; in: *Current topics in Plant Sciences*, (ed. J E Gunckel (New York: Acad. Press) pp 97–116
- Näf U 1979 Antheridiogens and antheridial development; in *The Experimental Biology of Ferns* (ed. A F Dyer (New York: Academic Press)
- Näf U, Nakanishi N and Endo M 1975 On the physiology and chemistry of fern antheridiogens; *Bot. Rev.* **41** 315–359

- Näf U, Sullivan J and Cummine M 1969 New antheridiogen from *Onoclea sensibilis*; *Science* **163** 1357–1360
- Nakanishi K, Endo M and Näf U 1971 Structure of the antheridium inducing factor of the fern *Anemia phyllitidis*; *J. Am. Chem. Soc.* **93** 5579–5581
- Pringle R B 1961 Chemical nature of antheridiogen-A, a specific inducer of the male sex organ in certain fern species; *Science* **133** 284
- Raghavan V 1976 Gibberellic acid-induced germination of spores of *Anemia phyllitidis*: Nucleic acid and protein synthesis during germination; *Am. J. Bot.* **63** 960–972
- Raghavan V 1977 Gibberellic acid-induced germination of spores *Anemia phyllitidis*: Autoradiographic study of the timing and regulation of nucleic acid and protein synthesis in relation to cell morphogenesis; *J. Cell Sci.* **23** 85–100
- Schedlbauer M D 1974 Biological specificity of the antheridiogen from *Ceratopteris thalictroides* (L.) Brongn; *Planta* **116** 39–43
- Schedlbauer M D 1976 Fern gametophyte development: Controls of dimorphism in *Ceratopteris thalictroides*; *Am. J. Bot.* **63** 1080–1087
- Schedlbauer M D and Klekovski E J 1972 Antheridiogen activity in the fern *Ceratopteris thalictroides* (L.) Brongn; *Bot. J. Linn. Soc.* **65** 399–413
- Schraudolf H 1962 Die Wirkung von Phytohormonen auf Keimung und Entwicklung von Farnprothallien. I. Austosung der Antheridien-bildung und Dunkelkeimung bei Schizaeaceen durch Gibberellinsäure; *Biol. Zentralbl.* **81** 731–740
- Voeller B R 1966 Gibberellins and growth in ferns; in *Proc. Internat. Symp. on Plant Stimulation, Sofia, Bulg. Acad. Sci.* pp 247–258
- Voeller B R 1971 Developmental physiology of fern gametophytes: Relevance for biology; *Bioscience* **21** 266–270
- Weinberg E S and Voeller B R 1969 Induction of fern spore germination; *Proc. Natl. Acad. Sci. USA* **64** 835–842

Nitrate reduction and assimilation in rice plants (*Oryza sativa* L.)

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Abstract. Nitrogen assimilation was studied in two rice varieties, Taichung Native 1, a high nitrogen tolerant strain and Ponni, a moderate nitrogen feeder. Nitrate and ammonia were supplied to the seedlings of the two strains at 3 levels 21, 42 and 84 $\mu\text{g-N/ml}$ of culture medium. Assimilation of nitrate and ammonia was followed by estimating levels of nitrate and nitrite reductases, glutamine synthetase and glutamate dehydrogenase activities. Growth, total nitrogen content, nitrate content and amino acid pool pattern were also determined at different age levels. It turned out that the high nitrogen tolerant variety T(N)1, most probably gets its nitrate reduction and ammonia assimilation done in the leaves with the photosynthetically generated reductant and energy, whereas in Ponni, much of these processes seem to occur in the roots at the expense of translocated photosynthates. Ammonia assimilation is primarily via GS/GOGAT pathway rather than via GDH and T(N)1 was higher in its assimilatory efficiency than Ponni.

Keywords. Nitrate reduction; GS/GOGAT pathway; Taichung Native 1; Ponni; nitrate reductase inactivating factor.

1. Introduction

Crop productivity during the last 50 years is highly correlated with fertilizer-N input. Maximised efficiency of use of soil and fertilizer-N is one of the main concerns of the fertilizer input technology and research imperatives directed towards this goal have often been emphasized.

Nitrogen utilization efficiency at the plant level involves a number of steps, such as uptake of nitrogen from the soil, reduction of nitrate and/or biosynthesis of amino acids in either root or shoot, transport of either reduced or unreduced forms of nitrogen, biosynthesis of enzymes involved in the processes and their regulation, compartmentation and remobilization of storage nitrogen. It is thus obvious that the problem is a complex one and involves the integration of all these aspects.

Nitrate reductase (NR) (EC 1.6.6.1) is the first enzyme in the assimilation path of nitrate—the predominant form of nitrogen available for crop plants in the field. Significant positive correlation exists between this enzyme and the nitrogen status of some higher plant systems, and that growth, yield or protein content are sometimes correlated with this enzyme level in seeds or leaves (Hageman 1979; Srivastava 1980).

Recently, Arima and Kumazawa (1975) have shown by the [^{15}N]-tracer method that glutamine is a primary product of ammonium assimilation and is synthesized from glutamic acid and newly absorbed ammonium by the catalytic activity of the enzyme glutamine synthetase (GS) (EC 6.3.1.2). The synthesis of [^{14}C]-glutamine from [^{14}C]-glutamate was differentially affected by the source of the nitrogen (Iyer *et al* 1981) and GS activity is reversibly repressed with increase in the external ammonium concentration (Rhodes *et al* 1975; Arima *et al* 1976). On the other hand, the universal existence of

physiological significance of this enzyme is still not clear, especially as regards its high K_m to ammonia and inhibition by ATP.

A comparison of the nitrogen assimilation efficiencies of two strains of paddy namely Taichung Native I (T(N)1), a high nitrogen tolerant variety and Ponni (Mashuri) which is only a moderate feeder, has been studied by growing them on three levels of nitrogen equivalents of nitrate and ammonium sources, 42 $\mu\text{g N/ml}$ being the optimum level and two other levels, one suboptimal and the other twice the normal level, representing high nitrogen level. Ammonium grown plants were included to see whether its assimilation proceeds *via* glutamine synthetase/glutamate synthase (GS/GOGAT) pathway or *via* glutamate dehydrogenase and also to indicate the tissue where it occurs primarily. Furthermore, ammonium fertilizers are usually favoured for rice culture.

2. Materials and methods

Rice plants were raised in culture solutions as per the procedure of Yoshida *et al* (1976). Seeds of T(N)1 and Ponni were obtained from the Paddy Experimental Station, Aduthurai, Tamil Nadu. The composition of the culture medium was as stated by Marwaha and Juliano (1976) and the source of nitrogen was from KNO_3 or $(\text{NH}_4)_2\text{SO}_4$, supplied in 3 different concentrations, namely 21, 42 and 84 $\mu\text{g N/ml}$. Rice plants were grown for the required duration in the green house under conditions of broad day light (8 K lux) and the pH of the culture medium was adjusted daily to 5.0.

Cell-free enzyme extracts were prepared from leaves and roots of 3, 5, 7, 10 and 12 days-old seedlings by grinding them separately at 4°C in a pre-chilled corning glass mortar and pestle using 0.01 M K-phosphate-KOH buffer (pH 7.5), containing 5 mM cysteine with 1:4 (W/V) ratio of the tissue to the extraction medium. The homogenate was squeezed through 8 layers of muslin cloth and centrifuged at 18,000 g for 30 min. The resulting clear supernatant was employed as crude enzyme source.

Nitrate reductase was assayed by the method of Hageman and Flesher (1960) as used by Marwaha and Juliano (1976). The reaction mixture (2 ml) contained in μmol : K-phosphate-KOH buffer, 100 (pH 7.5); KNO_3 , 20; NADH, 0.68, and shoot extract, 0.2 ml or root extract, 0.3 ml.

Nitrite reductase (NiR) (EC 1.6.6.4) was assayed by the method of Joy and Hageman (1966) with a slight modification as employed by Marwaha and Juliano (1976). The assay mixture (2 ml) contained in μmol : K-phosphate-KOH buffer, 100 (pH 7.5); NaNO_2 , 1.5; methyl viologen, 0.6; $\text{Na}_2\text{S}_2\text{O}_4$, 7.5 and shoot extract, 0.2 ml or root extract, 0.3 ml.

In suitable aliquots, the quantity of nitrite was determined with sulphanilamide-N-1-naphthylethylene diamine reagent at 540 nm. Enzyme activity was expressed as n mols of NO_2^- formed or consumed/15 min/mg protein.

Glutamine synthetase activity was measured by a modification of the procedure of Elliott (1953) in which the production of γ -glutamyl hydroxamate was measured colorimetrically (Shapiro and Stadtman 1970). The incubation mixture (3 ml) contained 0.2 M Tris-HCl buffer (0.5 ml, pH 7.5); 50 mM ATP (0.2 ml, pH 7.0); 0.5 M sodium glutamate (0.5 ml; pH 7.5); 0.1 M NH_2OHCl (0.3 ml, pH 7.5); 0.1 M cysteine (0.1 ml, pH 7.5); 0.1 M $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (0.1 ml) and shoot extract (0.3 ml) or root extract

terric chloride reagent (Shapiro and Stadtman 1970; Ferguson and Sims 1971) and absorption was read at 540 nm against a reagent blank devoid of ATP. Specific activity was expressed as n mols of γ -glutamyl hydroxamate formed/15 min/mg protein.

Glutamate dehydrogenase (GDH) (EC 1.4.1.4) was assayed by the method of Bulen (1956) as followed by Perez *et al* (1973). The enzyme activity was measured by following the initial rate of decrease in absorbance at 340 nm due to the oxidation of NADH to NAD at 25°C in a UNICAM sp 700 B recording spectrophotometer.

The reaction mixture (3 ml) contained 0.2 M K-phosphate-KOH buffer (2.0 ml, pH 8.0); 1.67×10^{-4} M NADH (0.5 ml, pH 8.0); 1.33×10^{-2} M α -Ketoglutarate (0.2 ml, pH 8.0); 0.067 M $(\text{NH}_4)_2\text{SO}_4$ (0.2 ml, pH 8.0) and enzyme extract from shoot or root (0.1 ml). Specific activity was expressed as n mols of NADH oxidised/15 min/mg protein.

Hundred milligrams of dried plant material or 1 g of fresh tissue (shoots and roots of 5 day-old rice seedlings) was boiled for 5 min in 5 ml of distilled water and then ground in a mortar. The aqueous extract was made up to 20 ml with distilled water and nitrate was estimated as outlined by Woolley *et al* (1960).

Total nitrogen content of shoots and roots was estimated by the modified micro-kjeldahl method (nesslerisation) (Umbreit *et al* 1972).

Amino acids from shoots and roots of 5 day-old rice seedlings were resolved by 2-dimensional thin layer chromatography on silica gel-G as described by Brenner and Nieder-wieser (1967). The extraction of amino acids was done in 80% (v/v) ethanol and after drying, the residue was dissolved in 1 ml of 10% (v/v) isopropanol. The supernatant was used for the amino acid analysis.

Protein was determined following the procedure of Lowry *et al* (1951). Bovine serum albumin (BSA) was used as the standard.

Assays were always run in triplicate and average of two concurrent values expressed. Experiments were repeated at least once and values were found to be reasonably close. Controls and blanks were run with the experiments as usual.

3. Results

The roots and shoots of T(N)1 registered an increase in nitrate content with increase in nitrate concentration of the culture medium (table 1). But, in the shoots of Ponni it showed an opposite trend. Maximum nitrate content was observed when it was raised in 42 $\mu\text{g/ml}$ nitrate-N. Further increase brought about decline in the nitrate content.

Table 1. Nitrate content of shoot and root of T(N)1 and Ponni grown in nitrate for a duration of 5 days.

N-concentration in $\mu\text{g/ml}$	Nitrate in $\mu\text{g}/100\text{mg}$ dry weight of material*			
	T(N)1		Ponni	
	Shoot	Root	Shoot	Root
21	3.73	183.3	172.0	212.2
42	276.8	402.7	167.7	487.2
84	526.2	483.2	128.2	259.3

* Average of six determinations.

21 $\mu\text{g/ml}$ nitrate-N. Conversely, roots of Ponni, reared in 84 $\mu\text{g/ml}$ nitrate-N, registered higher amount of nitrate than T(N)1. The shoots of T(N)1 cultured in ammonia had higher N content than those grown in nitrate. Similarly, shoots of Ponni reared in ammonia showed higher N content except the 3 and 10 day old plants grown in 42 $\mu\text{g/ml}$. Barring those of 5 day old plants grown in 21 $\mu\text{g/ml}$ nitrate-N and 7 day in 42 $\mu\text{g/ml}$ nitrate and ammonia-N, shoots of T(N)1 recorded higher N content than those of Ponni. At the highest level of nitrate supplied, T(N)1 roots accumulated higher quantity of N than those of Ponni, while at the lower levels, omitting 3 day old plants grown in 21 $\mu\text{g/ml}$ ammonia-N and 5 and 10 day in 42 $\mu\text{g/ml}$ nitrate and ammonia-N, Ponni had an edge over that of T(N)1 (tables 2 and 3).

Table 2. Total nitrogen content and specific activity of nitrate reductase, nitrite reductase, glutamine synthetase and glutamate dehydrogenase in shoots (S) and roots (R) of T (N)1 grown in NO_3^- and NH_4^+ at 21, 42 and 84 $\mu\text{g N/ml}$ of culture medium.

N Source	Concentration ($\mu\text{g N/ml}$)	Days germinated	Total N (mg/g dry wt.)		Enzyme activity in n moles of product formed or disappeared or oxidised/15 min/mg protein							
					NR		NiR		GS		GDH	
			S	R	S	R	S	R	S	R	S	R
NO_3^-	21	3	39	26	2	0	195	510	300	280	7.50	94.50
		5	42	17	5	0	225	255	600	610		
		7	49	20	8	0	300	225	520	450	7.35	127.50
		10	41	15	3	0	630	495	770	300		
	42	12	42	14	2	0	495	585	580	350	25.50	175.50
		3	48	24	69	0	195	315	540	240		
		5	70	30	91	0	150	225	580	290		
		7	32	16	44	0	105	285	540	180		
	84	10	63	21	32	0	195	585	700	180		
		12	46	16	2	0	255	270	630	210		
	21	3	51	30	158	63	120	585	820	430	8.40	96.00
		5	76	30	114	8	135	240	510	300		
		7	83	38	133	4	345	900	980	360	11.40	150.00
		10	58	27	4	0	210	210	760	290		
NH_4^+	21	12	74	32	9	0	270	330	670	1100	13.50	183.00
		3	49	32	0	0	75	30	260	570	6.60	75.00
		5	49	19	0	0	90	120	480	410		
		7	56	18	0	0	120	15	480	530	10.50	106.50
	42	10	55	17	0	0	195	750	750	540		
		12	44	12	0	0	150	555	600	440	24.00	156.00
	84	3	60	28	0	0	45	75	420	210		
		5	74	28	0	0	30	285	530	360		
		7	44	18	0	0	60	240	530	300		
		10	70	25	0	0	150	735	630	110		
	21	12	48	20	0	0	30	105	570	150		
		3	58	28	0	0	90	300	590	380	9.60	9.00
		5	79	28	0	0	75	330	510	520		
		7	85	61	0	0	90	675	740	500	14.25	69.00
	84	10	71	29	0	0	30	345	760	450		
		12	78	37	0	0	15	360	640	1200	15.00	106.50

Table 3. Total nitrogen content and specific activity of nitrate reductase, nitrite reductase, glutamine synthetase and glutamate dehydrogenase in shoots (S) and roots (R) of Ponni grown in NO_3^- and NH_4^+ at 21, 42 and 84 $\mu\text{g N/ml}$ of culture medium.

N Source	Concentration ($\mu\text{g N/ml}$)	Days germinated	Total N (mg/g dry wt.)		Enzyme activity in n moles of product formed or disappeared or oxidised/15 min/mg protein							
					NR		NiR		GS		GDH	
			S	R	S	R	S	R	S	R	S	R
NO_3^-	21	3	32	26	3	0	375	675	200	190	14-10	72-00
		5	46	28	13	0	240	330	380	290		
		7	32	22	9	0	255	420	360	290	16-35	174-00
		10	35	19	2	0	270	105	560	500		
	42	12	38	18	1	0	285	360	530	210	31-20	175-50
		3	39	35	10	0	250	315	150	140		
		5	49	16	21	0	255	540	480	140		
		7	42	25	22	0	240	360	690	250		
	84	10	46	16	3	0	300	345	620	280		
		12	41	18	2	0	315	675	860	400		
		3	49	30	19	5	15	165	270	230	12-00	100-50
		5	51	28	73	0	195	270	520	150		
		7	53	29	46	0	225	405	450	130	16-65	63-00
		10	27	19	15	0	150	450	660	80		
	21	12	39	21	7	0	480	735	530	60	16-80	178-50
		3	39	26	0	0	165	195	190	360	11-55	90-00
		5	46	32	0	0	30	45	340	400		
		7	41	26	0	0	15	45	440	660	15-30	160-50
NH_4^+	42	10	44	20	0	0	15	60	525	580		
		12	39	19	0	0	45	285	580	600	30-15	160-50
		3	35	31	0	0	15	45	170	480		
		5	49	20	0	0	45	75	380	550		
		7	46	27	0	0	15	15	560	790		
	84	10	44	18	0	0	45	585	620	330		
		12	44	20	0	0	45	450	1000	380		
		3	55	36	0	0	0	60	270	380	22-65	100-50
		5	66	30	0	0	15	210	420	500		
		7	56	30	0	0	120	390	540	380	28-35	49-50
		10	39	23	0	0	60	315	600	130		
		12	51	23	0	0	225	390	640	210	25-95	158-50

In both T(N)1 and Ponni, an increase in shoot NR activity was observed with increase in nitrate concentration of the culture medium. On the contrary, ammonia grown rice seedlings showed no NR activity. Also, with an increase in the concentration of nitrate-N from 21 to 42 $\mu\text{g/ml}$, the shoot NR activity in T(N)1 registered a 8 fold increase whereas in Ponni, it increased only 1 fold (tables 2 and 3). Maximum shoot NR activity was observed on the 5 day for T(N)1 and on 7 day for Ponni. At 84 $\mu\text{g/ml}$ nitrate-N, shoot NR activity increased 4 fold in Ponni and several fold in T(N)1. For T(N)1, the shoot NR activity was 225 nmoles product formed per 15 min per mg protein at 84 $\mu\text{g/ml}$ nitrate-N on 12th day. In Ponni, it was 380 nmoles product formed per 15 min per mg protein at 42 $\mu\text{g/ml}$ nitrate-N on 7th day. The root NR activity was very low in both T(N)1 and Ponni. It was 15 nmoles product formed per 15 min per mg protein in T(N)1 at 21 $\mu\text{g/ml}$ nitrate-N on 12th day. In Ponni, it was 15 nmoles product formed per 15 min per mg protein at 21 $\mu\text{g/ml}$ nitrate-N on 12th day. The root NR activity was very low in both T(N)1 and Ponni. It was 15 nmoles product formed per 15 min per mg protein in T(N)1 at 21 $\mu\text{g/ml}$ nitrate-N on 12th day. In Ponni, it was 15 nmoles product formed per 15 min per mg protein at 21 $\mu\text{g/ml}$ nitrate-N on 12th day.

inactivating factor in the roots of rice plants was already documented (Kadam *et al.* 1974; Yamaya and Ohira 1978) and we have also recorded the occurrence of a proteinaceous NR-inactivating factor in the roots of these rice varieties (unpublished data).

During extraction, the addition of chemicals that increases NR activity in other plants—PVP, at 50 % of the sample, which can bind phenols (Wallace 1973) or 1 or 3 % BSA (Schrader *et al.* 1974) did not enhance NR activity of either the shoot or root of these rice varieties.

Nitrite reductase activity was higher in rice seedlings grown in nitrate than in ammonia. Strangely, NiR activity was higher in roots than in shoots (tables 2 and 3). Taichung Native 1 recorded higher shoot NiR activity when grown in 21 $\mu\text{g/ml}$ nitrate-N, while Ponni showed higher shoot NiR activity at 42 $\mu\text{g/ml}$ nitrate-N. However, when cultured in 84 $\mu\text{g/ml}$ nitrate-N, T(N)1 edged over that of Ponni, barring the 5 and 12 days. In general, the development of shoot NiR activity kept pace with the greening process.

At 21 $\mu\text{g/ml}$ nitrate-N and at the early stages of growth, roots of Ponni showed higher NiR activity, while T(N)1 registered higher activity in the later stages (tables 2 and 3). At 42 $\mu\text{g/ml}$ nitrate-N also root NiR activity was higher in Ponni, except on the 10 days. There were fluctuations in the level of root NiR activity of T(N)1 raised in 84 $\mu\text{g/ml}$ nitrate-N, but in the roots of Ponni the NiR activity, though less than T(N)1, increased with increase in age. Regardless of the levels, ammonia reared T(N)1 exhibited higher root NiR activity than Ponni, the peak of activity occurring on the later stages of growth. The level of NiR was several fold higher than NR and did not seem to be limiting. Nitrite reductase activity was not inhibited, rather promoted by ammonia.

Glutamine synthetase activity in the shoots of T(N)1 was higher than that of Ponni except at 42 $\mu\text{g N/ml}$. At this level, on the 7 and 12 days, Ponni recorded higher GS activity than T(N)1. Nitrate at all levels, favoured higher GS activity than ammonia. However, GS activity in the roots was higher in ammonia grown seedlings. At 21 and 42 $\mu\text{g/ml}$ ammonia-N, GS activity in the roots of Ponni was higher whereas at 84 $\mu\text{g/ml}$ T(N)1 roots registered higher activity. Though not linear, increase in N concentration brought about increase in GS activity in the shoots of both the rice varieties, while, in the case of roots of Ponni, increase in nitrate concentration of the culture medium brought about a decrease in the GS activity (tables 2 and 3). Glutamine synthetase activity in roots of T(N)1 decreased when the concentration of the culture medium increased from 21 to 42 $\mu\text{g/ml}$ nitrate-N. Further raise to 84 $\mu\text{g/ml}$, resulted in an increase of activity to the extent of 5 fold. With regard to increase in ammonia-N concentration, GS activity in roots of T(N)1 followed a similar trend of decrease and increase, while in Ponni roots activity increased with increase in concentration from 21 to 42 $\mu\text{g/ml}$. Further increase brought about precipitous decline in the activity (table 3).

In the shoots, nitrate favoured higher GDH activity than ammonia, when the concentration was lowest. At the highest concentration it was *vice versa*. Ponni shoots reared in 21 $\mu\text{g/ml}$ nitrate-N showed higher GDH activity than T(N)1. At the highest level of ammonia-N, it was again Ponni that surpassed T(N)1 with regard to its GDH activity. Regardless of the source of N, it was shoots of Ponni that exhibited higher GDH activity than that of T(N)1 (tables 2 and 3).

Table 4. Intracellular amino acid pool patterns in shoots of T(NI) and Ponni.

Amino acids in $\mu\text{g/g}$ fresh weight	N-concentration in $\mu\text{g/ml}$											
	T(NI)						Ponni					
	21	42	84	21	42	84	21	42	84	21	42	84
	NO_3^-	NH_4^+	NO_3^-	NH_4^+	NO_3^-	NH_4^+	NO_3^-	NH_4^+	NO_3^-	NH_4^+	NO_3^-	NH_4^+
Alanine	2.2	1.9	15.1	8.9	2.1	3.3	11.1	15.5	1.9	46.6	1.9	4.4
Arginine	4.0	8.1	37.5	6.1	5.1	8.1	4.0	12.2	1.0	—	—	—
Aspartic acid	18.0	54.0	64.8	63.0	45.0	67.5	72.0	32.0	18.0	54.0	28.0	97.0
Asparagine	91.0	404.0	264.0	313.0	127.0	448.0	454.0	505.0	50.5	76.0	50.5	404.0
Glutamic acid	22.2	2.2	36.0	24.4	13.2	6.6	31.1	17.1	7.9	6.6	5.3	23.5
Glutamine	56.1	10.3	5.1	11.25	15.4	13.0	61.8	56.6	3.8	30.9	9.3	20.8
Glycine	14.1	19.2	60.8	4.0	9.6	12.0	38.4	29.2	7.1	14.1	5.7	10.4
Histidine	—	33.0	—	—	—	—	—	—	—	—	—	13.1
α -Aminobutyric acid	1.7	2.1	12.3	—	3.7	1.1	8.6	10.8	—	—	—	—
Leucine	1.0	3.0	4.2	2.6	6.5	3.9	7.9	—	3.9	—	1.0	2.0
Lysine	6.8	17.0	10.9	6.8	5.0	3.3	6.8	37.3	1.6	—	1.3	7.5
Valine	1.7	6.1	5.9	1.7	2.6	0.8	3.4	8.7	3.9	—	—	—
Total	218.8	560.9	516.6	441.75	235.2	567.6	699.1	723.4	99.6	228.2	103.0	582.7

— Not detectable

Glutamate dehydrogenase activity was higher in roots than in shoots of both the rice varieties. Roots of nitrate grown seedlings recorded higher GDH activity than those grown on ammonia. Except at the highest level of nitrate-N, where T(N)1 roots exhibited higher GDH activity (table 2), at all other levels of nitrate-N and ammonia-N, Ponni roots registered higher GDH activity (table 3). Amino acid analysis (tables 4 and 5) indicated that major differences in the composition was in the extremely high level of asparagine in the ammonia grown seedlings of both the strains. Yoneyama and Kumazawa (1974, 1975) also reported high asparagine content in rice seedlings raised in ammonia than in nitrate, but its turn over rate was very slow indicating that it was mainly a storage form of N. Shoots of T(N)1 showed greater amounts of asparagine than the roots, but in Ponni it was *vice-versa*. Of the 3 levels of N employed, maximum quantity of asparagine was registered when Ponni seedlings were raised in 21 $\mu\text{g/ml}$ ammonia-N. On the contrary, in shoots and roots of T(N)1, higher level of asparagine was found when seedlings were grown in 84 and 42 $\mu\text{g/ml}$ ammonia-N, respectively.

The level of glutamine was much less than that of asparagine. Shoots of Ponni cultivated in ammonia contained greater amounts of glutamine than those grown in nitrate, except at the lowest level. But in T(N)1, barring those that were grown in 42 $\mu\text{g/ml}$, glutamine was higher in shoots of seedlings grown in nitrate than those raised in ammonia. In both the varieties of rice, roots recorded higher levels of glutamine than shoots. Ponni roots raised in ammonia contained higher amounts of glutamine than those grown in nitrate, whereas it was the otherway about in T(N)1. Shoots in general, contained greater amounts of glutamic acid than roots. It was present in higher concentration in nitrate-grown shoots of both the rice varieties, except in shoots of Ponni raised in 84 $\mu\text{g/ml}$ ammonia-N. Roots of Ponni reared in ammonia recorded higher level of glutamic acid than those grown in nitrate except at 84 $\mu\text{g/ml}$ nitrate-N. In T(N)1 shoots, the opposite trend was observed.

4. Discussion

Nitrate reductase is the rate limiting enzyme in the biochemical reduction of nitrate to ammonia (Beevers and Hageman 1969). Since it is an inducible enzyme (Hageman and Flesher 1960; Beevers *et al* 1965), its activity increased in the presence of nitrate and ammonia completely repressed the enzyme synthesis (Pate 1973; Oaks *et al* 1977). The response of the two rice varieties to nitrate supply with respect to the development of shoot NR activity was significantly different. The leaves of T(N)1 showed a 8 fold increase in NR activity when supplied with 42 $\mu\text{g/ml}$ nitrate-N, while there was 1 fold increase only in the case of Ponni leaves (tables 2 and 3). Ponni performed better at lowest concentration of nitrate-N, while T(N)1 exhibited several fold increase at highest concentration. Not only the NR activity in T(N)1 was several fold higher, but the development of very high activity was evident even on the 3 day (table 2).

Roots of Ponni showed almost no NR activity that of T(N)1, when grown in 84 $\mu\text{g/ml}$ nitrate-N registered some activity on the 3 day only (table 2). Interference in the isolation of NR is less in young plant materials and this probably may account for the recovery of activity in root tissue at 3 day but not later. Further, the NR-inactivating factor reported to be present in the roots of rice seedlings (Kadam *et al* 1974; Yamaya

Table 5. Intracellular amino acid pool patterns in roots of T(N)1 and Ponni.

Amino acids in µg/g fresh weight	N-Concentration in µg/ml											
	T(N)1						Ponni					
	21	42	84	21	42	84	21	42	84	21	42	84
	NO ₃ ⁻	NH ₄ ⁺	NO ₃ ⁻	NH ₄ ⁺	NO ₃ ⁻	NO ₄ ⁺	NO ₃ ⁻	NH ₄ ⁺	NO ₃ ⁻	NO ₃ ⁻	NH ₄ ⁺	NH ₄ ⁺
Alanine	3.1	8.9	8.9	7.7	15.0	10.5	6.2	12.4	1.0	5.3	10.3	19.3
Arginine	2.0	-	9.6	-	-	3.0	2.8	-	4.0	1.5	19.2	4.0
Aspartic acid	25.0	9.0	42.0	81.0	21.6	27.0	25.0	37.0	22.5	25.0	20.0	30.0
Asparagine	50.5	127.0	140.0	234.0	50.5	192.0	284.0	568.0	50.5	61.0	454.5	303.0
Glutamic acid	4.6	2.2	5.2	2.5	6.2	9.5	3.1	6.2	3.8	3.5	5.2	3.0
Glutamine	35.7	23.1	34.0	18.0	9.3	2.5	21.5	72.1	6.4	5.9	72.3	11.1
Glycine	18.6	7.1	18.0	22.2	10.2	16.8	6.7	33.1	7.1	4.7	38.4	28.2
Histidine	-	-	-	-	-	-	-	-	-	-	-	-
α-Aminobutyric acid	3.2	6.4	10.0	-	7.7	5.4	3.0	12.1	1.7	19.8	19.8	5.0
Leucine	1.9	2.6	12.0	-	3.1	1.3	1.8	3.6	-	1.0	6.0	1.6
Lysine	7.1	3.3	8.0	11.9	4.0	2.5	4.7	14.2	1.9	1.2	15.6	10.9
Valine	2.4	1.7	8.1	-	1.7	1.7	2.4	2.4	-	-	8.0	-
Total	154.1	191.3	305.8	377.3	129.3	272.2	361.2	761.1	98.9	111.7	669.3	413.1

- Not detectable.

incorporated. Nitrate reduction and assimilation if taking place in the leaves utilizing photosynthetically generated reductant and energy would be advantageous to the plant as it is a highly energy-demanding process (Raven 1976).

Since NiR activity was several fold higher than NR activity, particularly in roots, this enzyme was neither rate-limiting nor was it inhibited by ammonia. On the contrary, ammonia enhanced the enzyme activity as reported by Mifflin (1974). Ponni roots in general, exhibited higher NiR activity than that of T(N)1 and such a high level of activity was suggestive of NR activity also, which perhaps was masked by the presence of NR-inactivating factor reported to be present in the roots. Higher level of NiR activity in the shoots of T(N)1 as against that of Ponni was in accordance with the observed higher level of shoot NR activity in T(N)1, and this as per Marwaha and Juliano (1976) reflects the relatively higher rate of oxidation of ammonia in the leaves.

Higher activity of GS in the shoots of both the rice varieties (tables 2 and 3) regardless of the form of nitrogen probably is an indication of GS/GOGAT pathway being a preferred mode of assimilation of ammonia-N. Taichung Native 1 appeared to be more efficient than Ponni. The higher tolerance for nitrogen in the former most probably was due to the fact that most of its inorganic nitrogen assimilation activities were performed in the shoots at the expense of photosynthetically generated reductant and energy.

Glutamate dehydrogenase activity was higher in roots than in shoots of both the rice varieties in both the sources of nitrogen. Ponni registered higher GDH activity than T(N)1. A relatively prominent role of GDH was ascribed when plants were grown in excessive concentration of ammonia (Barash *et al* 1973; Shepard and Thurman 1973). In the roots of Ponni, GDH activity was stimulated when the seedlings were exposed to higher levels of ammonia (table 3), and this brought about a steep decline in the GS activity. The repression of NR in the roots of Ponni and a parallel repression of GS with increased ammonia supply appeared to be associated with a switch over of ammonia assimilation from *via* glutamine to assimilation *via* glutamate. This was an indication of the poor efficiency of Ponni with regard to nitrogen utilization which appeared to be confined to the roots and hence energetically less economical from the point of view of the energy budget of the plant.

Analysis of the intracellular amino acid pool indicated a pattern consistent with the conclusions drawn. Glutamine level in roots of T(N)1, with increasing ammonia supply decreased, while a high level of glutamine was noticeable in the roots of Ponni. In the case of Ponni shoots, increase in nitrate supply caused a precipitous decline in glutamine level, increasing ammonia resulted only in a gradual reduction (table 4). This is in consistent with the pattern of nitrogen assimilation in the roots and transportation of reduced nitrogen in the form of glutamine to the above ground parts in Ponni. Similarly, greater amounts of asparagine was recorded in the shoots of T(N)1, whereas Ponni roots contained higher levels of this amide.

From the distribution of NR, NiR, GS and GDH activities and total nitrogen, nitrate and amino acid contents in the two rice varieties, it could be concluded that nitrogen assimilation in T(N)1 occurs predominantly in the leaves while it seems to take place in the roots in Ponni. Nitrogen assimilation when confined to the roots deprives the plant of the option of the direct use of photo-produced co-factors rather than respiration-derived co-factors, which could be uneconomical from the point of view of the energy budget of the plant (Raven 1976). Nitrogen assimilation also involves availability of carbon skeleton for synthesis of nitrogen containing organic assimilates. These are

From the comparison of these two rice varieties, it appears that compartmentation of nitrogen assimilation reactions in the leaves could confer greater efficiency of nitrogen utilization and thus might account for the higher nitrogen tolerance of T(N)1. This not only imposes a pH stress in the shoots (Raven and Smith 1976; Smith and Raven 1979; Pate 1980), but also competition for the photo-produced energy and reductant for carbon fixation and nitrogen assimilation and these have to be modulated without sacrificing the efficiency of one or the other.

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References

- Arima Y and Kumazawa K 1975 A kinetic study of amide and amino acid synthesis in rice seedling roots fed with ^{15}N labelled ammonium (part 2). Physiological significance of glutamine on nitrogen absorption and assimilation in plants; *J. Sci. Soil. Manure. Jpn.* **46** 355-361
- Arima Y, Horinouchi T and Kumazawa K 1976 Variation and regulation of glutamine synthetase activity in rice seedlings fed with ammonium and nitrate (part 4). Physiological significance of glutamine on nitrogen absorption and assimilation in plants; *J. Sci. Soil and Manure Jpn.* **47** 198-203
- Barash I, Sadon T and Mor H 1973 Induction of a specific isoenzyme of glutamate dehydrogenase by ammonia in oat leaves; *Nature (London) New Biol.* **244** 150-152
- Beevers L, Schrader L E, Flesher D and Hageman R H 1965 The role of light and nitrate in the induction of nitrate reductase in radish cotyledons and maize seedlings; *Plant Physiol.* **40** 691-698
- Beevers L and Hageman R H 1969 Nitrate reduction in higher plants; *Ann. Rev. Plant Physiol.* **20** 495-522
- Bulen W A 1956 The isolation and characterization of glutamic dehydrogenase from corn leaves; *Arch. Biochem. Biophys.* **62** 173-183
- Brenner M and Niederwieser A 1967 Thin-layer chromatography (TLC) of amino acids; *Methods Enzymol.* **11** 39-58
- Elliot W H 1953 Isolation of glutamine synthetase and glutamotransferase from green peas; *J. Biol. Chem.* **201** 661-672
- Ferguson A R and Sims A P 1971 Inactivation *in vivo* of glutamine synthetase and NAD-specific glutamate dehydrogenase; its role in the regulation of glutamine synthesis in yeasts; *J. Gen. Microbiol.* **69** 423-427
- Hageman R H 1979 Integration of nitrogen assimilation in relation to yield; in *Nitrogen Assimilation of Plants* (eds) E J Hewitt and C V Cutting (London: Academic Press) pp 591-611
- Hageman R H and Flesher D 1960 Nitrate reductase activity in corn seedlings as affected by light and nitrate content of nutrient media; *Plant Physiol.* **35** 700-708
- Iyer R K, Tuli R and Thomas J 1981 Glutamine synthetases from rice: Purification and preliminary characterization of two forms in leaves and one form in roots; *Arch. Biochem. Biophys.* **209** 628-636
- Joy K W and Hageman R H 1966 The purification and properties of nitrite reductase from higher plants and its dependence on ferredoxin; *Biochem. J.* **100** 263-273
- Kadam S K, Gandhi A P, Sawhney S K and Naik M S 1974 Inhibition of nitrate reductase in the roots of rice seedlings and its effect on the enzyme activity in the presence of NADH; *Biochim. Biophys. Acta* **350** 162-170
- Lowry O H, Rosebrough N J, Farr A L and Randall R J 1951 Protein measurement with the Folin phenol reagent; *J. Biol. Chem.* **193** 265-275
- Marwaha R S and Juliano B O 1976 Aspects of nitrogen metabolism in the rice seedlings; *Plant Physiol.* **57** 923-927

- Mifflin B J 1974 Nitrate reduction in leaves. Studies on isolated chloroplasts; *Planta* **116** 187-196
- Oaks A, Aslam M and Boesel I 1977 Ammonium and amino acids as regulators of nitrate reductase in corn roots; *Plant Physiol.* **59** 391
- Pate J S 1973 Uptake, assimilation and transport of nitrogen compounds by plants; *Soil Biol. Biochem.* **5** 109-119
- Pate J S 1980 Transport and partitioning of nitrogenous solutes; *Ann. Rev. Plant Physiol.* **31** 313-340
- Perez C M, Cagampang G B, Esmama B V, Monserrate R U and Juliano B O 1973 Protein metabolism in leaves and developing grains of rice differing in grain protein content; *Plant Physiol.* **51** 537-542
- Raven J A 1976 Transport in algal cells and tissues; in *Encyclopedia of plant physiology* (Volume on short distance transport) (eds) U Lüttge and M G Pitman (Berlin: Springer Verlag)
- Raven J A and Smith F A 1976 Nitrogen assimilation and transport in vascular land plants in relation to intracellular pH regulation; *New Phytol.* **76** 415-431
- Rhodes D, Rendon G A and Stewart G R 1975 The control of glutamine synthetase level in *Lemna minor* L; *Planta.* **125** 201-211
- Schrader L E, Cataldo D A and Peterson D M 1974 Use of protein in extraction and stabilization of nitrate reductase; *Plant Physiol.* **53** 688-690
- Shapiro B M and Stadtman E R 1970 Glutamine synthetase; *Methods Enzymol.* **17** 910-922
- Shepard D V and Thurman D A 1973 Effect of nitrogen sources upon the activity of L-glutamate dehydrogenase of *Lemna gibba*; *Phytochemistry* **12** 1937-1946
- Smith F A and Raven J A 1979 Intracellular pH and its regulation; *Ann. Rev. Plant Physiol.* **30** 289-311
- Srivastava H S 1980 Regulation of nitrate reductase activity in higher plants; *Phytochemistry* **19** 725-733
- Umbreit W W, Burris R H and Stanffer J F 1972 Methods for nitrogen; in *Manometric and Biochemical techniques*; 5th edn (Minnesota: Burgess Publishing Co.) pp 259-260
- Wallace W 1973 A nitrate reductase inactivating enzyme from the maize roots; *Plant Physiol.* **52** 197-201
- Woolley J T, Hicks G P and Hageman R H 1960 Rapid determination of nitrate and nitrite in plant material; *J. Agric. Food Chem.* **8** 481-482
- Yamaya T and Ohira K 1978 Nitrate reductase inactivating factor from rice seedlings; *Plant Cell Physiol.* **19** 211-220
- Yoneyama T and Kumazawa K 1974 A kinetic study of the assimilation of ^{15}N -labelled ammonium in rice seedling roots; *Plant Cell Physiol.* **15** 655-662
- Yoneyama T and Kumazawa K 1975 A kinetic study of the assimilation of ^{15}N -labelled nitrate in rice seedlings; *Plant Cell Physiol.* **16** 21-26
- Yoshida S D, Forno A, Cock J H and Gomez K A 1976 *Laboratory manual for physiological studies of rice*; 3rd edn (Las Banos, Philippines: International Rice Research Institute)

Environmental control of cell morphology in desmids

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Abstract. The variability of the desmids was quite interesting to note that when the conditions such as temperature and illumination were altered, there was not only an increase in the abnormal forms but the number of the adherent cells were also more. This abnormal behaviour of the species was more or less directly proportionate to the altered status of these factors in culture. It was thus quite clear that due to an increase or decrease in temperature and light intensity, quite a number of variants were produced. Sometimes the cells departed so widely from the specific characteristic that one could mistake them as belonging to different taxa. It may be that under unfavourable conditions of growth the mechanism of cell division was disturbed lowering thereby the percentage of mitosis and finally leading to the tendency of morphological aberrations. Consequently, these observations induced to study the various species of desmids under different experimental conditions to find out the range of morphological variation in cultures because the feature which more than any other, has attracted algologists to study desmids especially placoderms is their considerable morphological variability. Morphological variations under different cultural conditions have been studied and it is presumed that the cell types are capable of maintaining their narrow specificity, which is genetically controlled under favourable conditions only, but, whenever, there is a change in environmental set-up, it has resulted in upsetting the metabolic behaviour leading to abnormal forms.

Keywords. Desmids; morphology; ecology; pH; temperature; illumination.

1. Introduction

Andhra Pradesh, a South Indian State, is located at an altitude of 300–2,500 ft and with an average rain-fall 1143–1270 mm in the north and 508 mm in the south-west per year, offers very good collection spots for desmid species. The variability of the desmids has been pointed out by many earlier workers like West (1899), Duceillier (1915), Czurda (1926), Pringsheim (1930), Ondracek (1936), Lefevre (1939), Rosenberg (1944), Teiling (1956), Waris and Kallio (1957) and Nizam (1968). Recent studies of naturally occurring populations of various species of placoderm desmids have been undertaken by various investigators (Villeret 1951; Teiling 1956; Brook 1959a, b; Lind and Croasdale 1966; Brook and Hine 1966; Tyler 1971; Vidyavati and Nizam 1972; Bicudo 1975; Kirk and Cox 1975; Sathaiah 1983; Mogili and Vidyavati 1985).

2. Materials and methods

The clone dealt with here belongs to the specimen *Euastrum spinulosum*-Delp. var. *duplo-minor*, with a clone no. 260. The species was collected from rocky pan in the Osmania University Campus, and is preserved in the Department of Botany. Other two species were kindly supplied by Cambridge culture collection, UK. The clonal cultures thus raised, were the species—*Cosmarium botrytis* and *Cosmarium praemorsum*.

Stock cultures were maintained in biphasic medium on a rack placed in the north window at room temperature. Liquid cultures were established in 250 cc Pyrex conical flasks containing 150 cc autoclaved Waris solution.

Ten cc of the culture was centrifuged at 2000–2500 r.p.m. and after pouring out the supernatant liquid, the sedimented cells were added to a fresh 50 cc Waris medium, contained in 100 ml Pyrex conical flasks. The cultures of *E. spinulosum* were subjected to different experimental conditions:

- (i) Day light only at the north window at 21–37°C.
- (ii) Alternate light and dark period of 12 hr duration at 24–37°C.
- (iii) Continuously illuminated cabinet at 24–38°C.
- (iv) Continuously illuminated cabinet at 17–32°C.
- (v) 16 hr illumination alternated by 8 hr dark period at 16–30°C.
- (vi) Continuous illumination at 18–22°C.
- (vii) 16 hr light period and 8 hr dark period at 18–22°C.

The culture conditions for *C. botrytis* and *C. praemorsum* are given in the tables 2 and 3, respectively.

Among the environmental factors mostly pH, temperature and illumination conditions were studied.

3. Results

Observations were recorded every 5th day from all the samples. From each sample 50 randomly selected cells were measured for their length and breadth (tables 1–3). In the case of *E. spinulosum*, the highest percentage of the cells under all conditions were those having 43.5 μ m length and 34.8 μ m breadth, which is almost in close approximation with mean length (45.6 μ m) and breadth (38.5 μ m). These figures closely correspond with the length and breadth given for *E. spinulosum* by Krieger (1937), which

Table 1. Showing cell length and breadth under varied cultural conditions.
Euastrum spinulosum Delp. var. *duplo-minor* W. and W.

Culture condition		Length (μ m)	Breadth (μ m)
Day light at 21–37°C	Range	34.8–60.9	26.1–52.2
	Mean	48.0008	40.9074
Alternate light at 24–37°C	Range	34.8–78.3	26.1–52.2
	Mean	46.3942	39.1616
Constant light at 24–38°C	Range	34.8–113.1	26.1–52.2
	Mean	45.2690	38.2742
Alternate light at 16–30°C	Range	26.1–147.9	26.1–60.9
	Mean	45.7678	38.1988
Continuous light at 17–32°C	Range	26.1–174.0	17.4–52.2
	Mean	45.1356	36.8648
Alternate light at 18–22°C	Range	34.8–95.7	26.1–52.2
	Mean	46.8524	40.6640
Continuous light at 18–22°C	Range	26.1–121.8	17.4–52.2
	Mean	42.2298	35.4380

Culture condition		Length (μm)	Breadth (μm)
Alternate light at 18–22°C	Range	47.5–92.5	32.5–87.25
	Mean	74.586	63.258
Alternate light at 30–32°C	Range	47.5–92.5	32.5–79.5
	Mean	72.25	61.756
Constant light at 27–29°C	Range	47.5–87.25	32.5–75.0
	Mean	70.570	60.546
Day light at 27–29°C	Range	47.5–82.50	32.750
	Mean	76.850	66.540
Refrigerator cabinet with constant light at 8–9°C	Range	47.5–77.50	32.5–70.00
	Mean	70.00	57.428

Table 3. Showing cell length and breadth under varied cultural conditions. *Cosmarium praemorsum* Breb.

Culture condition		Length (μm)	Breadth (μm)
Alternate light at 18–22°C	Range	27.25–70.00	17.50–57.25
	Mean	52.887	43.435
Alternate light at 30–32°C	Range	27.25–70.00	17.50–57.25
	Mean	50.640	42.890
Constant light at 27–29°C	Range	27.25–62.50	17.50–57.25
	Mean	48.25	42.50
Day light at 27–29°C	Range	27.25–62.50	17.50–57.50
	Mean	54.75	48.25
Refrigerator cabinet with constant light at 8–9°C	Range	27.25–57.50	17.50–55.00
	Mean	46.50	37.780

incidentally vary from 42–80 μm and 38–73 μm , respectively. This is also more or less in accord with the measurements given by various authors for this species, after the publication of Krieger's work, and gathered from different parts of the world. In *C. botrytis* the highest percentage of cells under all conditions were those having 74.50 μm length and 61.50 μm breadth; which is almost close with the mean length 72.91 μm and breadth 63.50 μm . These figures approximately correspond to the length and breadth given for *C. botrytis* Menegh; by West and West (1912). In *C. praemorsum* the highest percentage of the cells under all conditions was those having 52.75 μm length and 43.75 μm breadth which is almost close with the mean length 52.8875 μm and breadth 43.4337 μm . These figures closely correspond to the length and breadth given for *C. praemorsum* Breb. by West and West (1908).

Apart from these culture studies, some environmental factors were also considered.

During periodic collections, the author could assess an overall important role of temperature on the periodicity and growth of desmids in the natural habitats. Desmid species were found to be relatively in abundance in early summer. During this period the water temperature varied from 26–28°C, increasing gradually as the months

affected, because they progressively diminished in number. It seems that the maximum temperature which they could tolerate in natural populations is somewhere around 38°C.

The data gathered during the periodic collection of desmid species, has clearly indicated that most of the species flourished well during the early summer months. It is presumed that for the present species the most favourable range of temperature was 18–22°C. Water temperature and prolonged periods of sunshine are considered to be favourable in the periodicity of desmids in general (Fritsch and Rich 1913; Hodgetts 1922; Rao 1955). Venkateswarlu (1983) has shown that water temperature and algal numbers showed a direct relationship and the highest peak reached at maximum temperature (33.5°C) in case of *Staurostrum tetracerum* Ralfs.

Desmids occur abundantly in acidic environments (Joshua 1886; West and West 1909; Strom 1924; Griffiths 1928; Froehne 1939), but Van Oye (1934) pointed out that the desmids increase with the rise in pH. Hutchinson *et al* (1932) found 30–40 species of desmids in South Africa at 9.0 pH. According to Rao (1955) there are few desmid species which are quite indifferent to pH variations. Venkateswarlu (1983) has observed *S. tetracerum* growing luxuriantly in alkaline water a pH range of 8.1–10.0.

For the optimum growth of these species various culture media with varied pH range were employed (5.25–8.0). The growth was estimated by optical density and cell count. The best growth was obtained in medium having pH 6.0–7.5; which corresponded more or less with those of the habitats from where the species were originally collected.

For artificial illumination, the test tubes were kept in a cabinet fitted with 40 Watts day light fluorescent tubes of 6500°K, and 15 cm distance from the light source. Desmids showed a tendency to attain a good growth under artificial illumination, where the light intensity was twice as much as the day light. Of all the illumination conditions tried, it is now well established that 16 hr light period alternated by 8 hr dark period is most suitable for the optimum growth of the desmids.

4. Discussion

The observations accumulated on the morphological variations of *E. spinulosum*, *C. botrytis* and *C. praemorsum*, under the present experimental conditions suggest that the specific definitions of the species tend to be much altered under unfavourable physical and physiological factors. The type is capable of maintaining its narrow specificity which is genetically controlled under the favourable conditions only and whenever there is a change in the environmental set-up, it results in upsetting the cell metabolism leading to the change in morphological patterns which vary according to the intensity of the various factors.

On this assumption it can be presumed that the species or varieties which are discriminated from each other on the basis of one or two differences may, at least, in some cases, belong to the same genetical stock and may not warrant placing them in two different taxa. Such types of experimental studies would certainly help in the elimination of certain taxonomic confusions, especially in Desmidiaceae, where small variations in the measurement of the cells have so often led to the creation of new species, varieties and forms.

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References

- Bicudo C E M 1975 Polymorphism in the desmid *Arthodesmus mucronulatus* and its taxonomic implications; *Phycologia* **14** 145–148
- Brook A J 1959a The status of Desmids in the plankton and the determination of phytoplankton quotients; *J. Ecol.* **47** 429–445
- Brook A J 1959b *Staurastrum paradoxum* Meyen. and *S. gracile* Ralfs. in the British freshwater plankton and a revision of the *S. anatinum*—group of radiate desmids; *Trans. R. Soc. Edinburgh* **58** 589–628
- Brook A J and Hine A E 1966 A population of *Staurastrum freemanii* from the Central Highlands of New Guinea; *J. Phycol.* **2** 66–73
- Czurda V 1926 über die Reinkultur von conjugacen; *Arch. Protistenkd.* **54** 355–358
- Ducellier F 1915 Contribution a l'Etude du polymorphisme et des Montrosites chez des Desmidiaceae; *Bull. Soc. Bot. Geneva* **3/4** 75–118
- Fritsch F E and Rich F 1913 Studies on the occurrence and reproduction of British freshwater Algae in nature. III. A four year's observation of a freshwater pond; *Ann. Biol.* **6** 1–83
- Froehne W C 1939 Anopheline breeding; suggested classification of ponds based on the characteristic desmids; *Public Health Rep.* **30** 1363–1387
- Griffiths B 1928 On desmid plankton; *New Phytol.* **27** 98–107
- Hodgetts W J 1922a A study of some of the factors controlling the periodicity of freshwater algae in nature; *New Phytol.* **21** 15–33
- Hutchinson G E, Pickford G E and Schuurman J F M 1932 A contribution to the hydrobiology of pans and other inland waters of South Africa; *Arch. Hydrobiol.* **24** 1–154
- Joshua W 1886 Burmese Desmidiaceae, with description of new species occurring in the neighbourhood of Rangoon; *J. Linn. Soc. London, Bot.* **21** 634–656
- Kirk W L and Cox E R 1975 Observations on polymorphism in the green algae *Cosmarium botrytis* Menegh. (Desmidiaceae); *Phykos* **14** 35–45
- Krieger W 1937 Die Desmidiaceen Europas mit Berücksichtigung der ausser europäischen Arten; *I. Teil* 633–634, 637–638
- Lefevre M 1939 Recherches experimentales sur le polymorphisme et la Teratologia des Desmidices; *Encyl. Biol. Paris* **19** 42
- Lind E M and Croasdale H 1966 Variation in the desmid *Staurastrum sebaldi* var. *ornatum*; *J. Phycol.* **2** 111–116
- Mogili T and Vidyavati 1985 Impact of varied culture conditions on growth and morphology of *Cosmarium granatum* Breb; *J. Swamy Bot. Club* **2** 33–42
- Nizam J 1968 Morphological variations in desmids with particular reference to *Cosmarium pyramidatum* Breb; *Osmania University Golden Jubilee Comm.* 139–146
- Ondracek K 1936 Experimentelle Untersuchungen über die Variabilität einiger Desmidiaceen; *Planta* **26** 226–246
- Pringsheim E G 1930 Die kultur von *Micrasterias* und *Volvox*; *Arch. Protistenkd.* **72** 1–48
- Pringsheim E G 1946 The biphasic or soil-water culture method for growing algae and flagellata; *J. Ecol.* **33** 193–204
- Rao C B 1955 On the distribution of algae in a group of six small ponds, II. Algal periodicity; *J. Ecol.* **43** 291–308
- Rosenberg M 1944 On the variability of the desmid *Xanthidium-subhastiferum* West; *New Phytol.* **43** 15–22
- Sathaiah G 1983 *Cytological and experimental studies in Cosmarium species*, Ph.D. thesis, Kakatiya University, Warangal
- Storm K M 1924 Studies in the ecology and geographical distribution of freshwater algae and plankton; *Rev. Algol.* **1** 127–155

- Teiling E 1956 On the variation of *Micrasterias mahabuleshwarensis* F. Wallich; *Bot. Not.* **109** 260–274
- Tyler P A 1971 A simple and rapid technique for surveying size and shape variation in desmids and diatoms; *Br. Phycol. J.* **6** 231–233
- Van Oye P 1934 Quelques donnees sur l'ecologie des Desmidiées; *Bull. Soc. R. Bot. Belg.* **67** 65–75
- Venkateshwarlu V 1983 Ecology of Desmids 1. *Staurostrum tetracerum* Ralfs; *Indian J. Bot.* **6** 68–73
- Vidyavati and Nizam J 1972 Morphological variations under varied cultural conditions with particular reference to *Euastrum spinulosum* Delp. var. *duplo-minor* W & W; *Phykos* **11** 10–16
- Villeret S 1951 L'déphasage des divisions cellulaires et de la Morphogenèse chez les Desmidiées unicellulaire; *Bull. Soc. Sci. Bretagne* **26** 49–58
- Waris H and Kallio P 1957 Morphogenetic effects of chemical agents and micro-cyto-plasmic relations in *Micrasterias*; *Ann. Acad. Sci. Fenn. A.* **37** 1–20
- West G S 1899 On variation in the Desmidiaceae; *J. Linn. Soc.* **34** 366
- West W and West G S 1908 *A Monograph of the British Desmidiaceae* (Cambridge: Cambridge University Press) Vol. 3, p. 196
- West W and West G S 1909 The British freshwater phytoplankton with special reference to the desmid-plankton and the distribution of British Desmids; *Proc. R. Soc. London Ser. B.* **81** 165–206
- West W and West G S 1912 *A Monograph of the British Desmidiaceae* (Cambridge: Cambridge University Press) Vol. 4

Effect of sulphur dioxide on growth and nodulation of pigeonpea

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Abstract. Effect of sulphur dioxide on the growth of pigeonpea (*Cajanus cajan* (L.) Millsp.) was studied. Five day old pigeonpea seedlings were exposed to 10 and 20 ppm SO₂ in glass chambers for 3 hr/day continuously for two weeks. Analyses were made in 25-day old seedlings with respect to foliar injury, morphological characteristics, dry matter accumulation and nodule number. Sulphur dioxide affected leaves showed chlorosis, tip burn and marginal necrosis. Root growth and nodulation were very much reduced in fumigated seedlings. Sulphur dioxide exposure decreased dry matter accumulation in all the parts of the seedling.

Keywords. Sulphur dioxide; *Cajanus cajan*; morphological characteristics; dry matter accumulation; nodulation.

1. Introduction

The impact of air pollutants on agricultural crops has not been assessed so far. Sulphur dioxide is one of the most common industrial air pollutants. It has been established that sulphur dioxide causes deleterious effects on plants (Thomas and Hendricks 1956; Ricks and Williams 1975; Pandey and Rao 1978). Sulphur dioxide enters the plant mostly through stomata and at toxic concentrations induces alterations in various biochemical and physiological processes leading to reduced growth (Thomas 1961; Thomas and Hendricks 1956; Rao and LeBlanc 1965; Coker 1967; Daines 1968; Syrratt and Wanstall 1969; Bell and Clough 1973; Ashenden 1978). Since most of the studies are based on their local general vegetation, as there is little work on its influence on crop plants and the degree of response and deleterious effects varies from plant to plant, the present investigation is concerned with the effect of high SO₂ concentrations of short term exposures on the growth and development of pigeonpea; an important pulse crop of India. These simulation studies may help in understanding the mode and mechanism of the deleterious effects of SO₂ on this crop plant.

2. Materials and methods

Pigeonpea (*Cajanus cajan* (L.) Millsp.) cv. PDM1 seeds were planted in pots (18 cm diameter) containing soil and farmyard manure in 3:1 ratio. For proper nodulation the seeds were washed and soaked in distilled water for 3 hr to remove water soluble Rhizobial growth inhibiting substances, if any from seed coats and then the seeds were inoculated with 7-day-old cultures of *Rhizobium* isolated from the same cultivar and grown in yeast mannitol agar broth. Since the *Rhizobia* that infect pigeonpea is a slow growing type (cowpea type) they require 6–14 days to form 1–2 mm colonies (Dye 1980). Therefore 7-day-old cultures were used. After 5 days thinning was done, keeping 10 plants per pot. Three pots were fumigated in each concentration of 10 and 20 ppm

control seedlings were kept in glass chambers without SO₂. The seedlings were always fumigated from 8-00 a.m. to 11-00 a.m. after which the plants were allowed to grow under normal conditions. Desired SO₂ concentrations in glass chambers (0.45 m³) were obtained by reacting Na₂SO₃ with dilute H₂SO₄ by the relationship that 2.6 mg of Na₂SO₃ with dilute H₂SO₄ releases 1 ppm SO₂ in 1 m³ air (Rao and Le Blanc 1965). The released SO₂ get mixed with air in the chamber and produces the desired SO₂ concentration. Analyses were made on 25-day-old seedlings.

Fresh weights of the control and fumigated seedlings were determined immediately on harvest. The materials were kept in hot air oven at 80°C until constant dry weights were obtained.

Shoot/root ratios were determined by the procedure of Evans and Hughes (1961) as presented in the following formulae:

$$\text{Shoot/root ratio (dry weight)} = \frac{\text{Mean shoot dry weight in mg}}{\text{Mean root dry weight in mg}} \cdot$$

The level of injury such as chlorosis and necrosis was expressed in qualitative terms.

3. Results and discussion

The symptoms, chlorosis, tip burn and marginal necrosis were observed in both concentrations, however, the damaging effect was more with 20 ppm than with 10 ppm SO₂.

The shoot length and root length recorded higher values for the control than the fumigated seedlings. Among the fumigated plants those received 20 ppm SO₂ showed lower values than those treated with 10 ppm SO₂. The root length was more affected than shoot length. The root length of seedlings exposed to 10 and 20 ppm SO₂ were reduced by 52.47 % and 61.15 % respectively on compared to the controls (table 1).

Nodule number was also reduced in pigeonpea seedlings due to SO₂ exposure. Control seedlings showed on an average 23.4 nodules per seedling, whereas 9.2 and 3.6 nodules per seedling were observed in 10 and 20 ppm SO₂ exposed seedlings respectively. The number of leaves per seedling were also reduced in the fumigated seedlings (table 1).

Depending on the level of SO₂ exposure a reduction in the fresh and dry weight of the

Table 1. Effect of sulphur dioxide on shoot length, root length, nodule number and leaf number of pigeonpea seedling (mean of 30 seedlings with SE)

SO ₂ con.	Shoot (cm)	Root (cm)	Nodule number	Number of leaves
0 ppm	26.43 ± 0.69	27.88 ± 1.83	23.40 ± 0.73	6.10 ± 0.17
10 ppm	17.44 ± 0.34	13.25 ± 0.62	9.20 ± 0.69	5.60 ± 0.16
20 ppm	16.65 ± 0.32	10.83 ± 0.62	3.60 ± 0.49	4.50 ± 0.16

	Sulphur dioxide concentration		
	0 ppm	10 ppm	20 ppm
<i>Fresh weight (mg)</i>			
Whole seedling	2470.43 ± 17.89	1255.22 ± 13.66	962.83 ± 1.12
Stem	544.99 ± 6.73	293.32 ± 1.26	240.41 ± 1.10
Leaf	1016.10 ± 4.33	689.99 ± 3.78	528.75 ± 1.25
Root	909.44 ± 6.75	271.90 ± 8.66	193.66 ± 0.79
<i>Dry weight (mg)</i>			
Whole seedling	353.93 ± 4.87	171.36 ± 1.57	154.11 ± 0.18
Stem	104.61 ± 1.97	48.71 ± 0.29	45.83 ± 0.54
Leaf	184.44 ± 1.69	100.71 ± 0.73	88.66 ± 0.29
Root	64.88 ± 1.29	21.94 ± 0.58	19.62 ± 0.57
Shoot/root ratio	1.60 ± 0.00	2.22 ± 0.07	2.33 ± 0.06

whole seedling as well as its different parts (stem, leaf and root) was observed. Of all the plant parts roots were much affected. The roots showed 66.18 % and 66.67 % decrease in dry weight in 10 and 20 ppm SO₂ exposed seedlings respectively over the controls. The stem growth showed 53.43 % reduction in 10 ppm and 56.18 % reduction in 20 ppm SO₂ exposed seedlings. The dry weights of the leaves of SO₂ fumigated seedlings showed 45.39 % and 51.93 % decrease in 10 and 20 ppm respectively. Since the root system of the fumigated seedlings were very much affected, the shoot/root ratio was increased in fumigated seedlings than the control seedlings (table 2).

The growth reduction may be due to the reduced photosynthetic area probably resulting in reduced photosynthesis (Rao and Le Blanc 1965; Cocker 1967). The reduced root growth and nodulation may be due to the reduced photosynthate being available for translocation to the roots (Ashenden 1978; Mejsrik 1980; Marshall and Furnier 1981; Jones and Mansfield 1982; Singh and Rao 1982).

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References

- Ashenden T W 1978 Growth reduction in Cocksfoot (*Dactylis glomerata* L.) as a result of SO₂ pollution; *Environ. Pollut.* **15** 161-166
- Bell J N B and W S Clough 1973 Depression of yield in rye grass exposed to sulphur dioxide; *Nature (London)* **241** 47-49
- Coker P D 1967 Effects of SO₂ pollution on the Bark epiphytes; *Trans. Br. Bryol. Soc.* **5** 341-347
- Daines R H 1968 Sulphur dioxide and plant response; *J. Occup. Med.* **10** 516-34
- Dye M 1980 Functions and maintenance of a Rhizobium collection in *Recent Advances in Biological Nitrogen Fixation* (ed) N S Subba Rao (Oxford and IBH Publishing Company New Delhi) pp 435-471
- Evans G C and A P Hughes 1961 Plant and the aerial environment. 1. The effect of artificial shading on *Impatiens parviflora*; *New Phytol.* **60** 150-180
- Jones T and T A Mansfield 1982 The effects of SO₂ on growth and development of seedlings of *Phleum pratense* under different light and temperature environments; *Environ. Pollut. Series (A)* **27** 57-71
- Marshall P E and G R Furnier 1981 Growth responses of *Alianthus altissima* seedlings to SO₂; *Environ. Pollut. Series (A)* **25** 149-153
- Mejstrik V 1980 The influence of low SO₂ concentrations on growth reduction of *Nicotiana tabacum* L. cv. samsun and *Cucumis sativus* L. cv. Unikat; *Environ. Pollut. Series (A)* **21** 73-76
- Pandey S N and D N Rao 1978 Effects of coal, smoke, sulphur dioxide pollution on the accumulation of certain minerals and chlorophyll content of wheat plants; *Trop. Ecol.* **19** 155-162
- Rao D N and F Le Blanc 1965 Effects of sulphur dioxide on lichen algae with special reference to chlorophyll; *Bryologist* **69** 69-75
- Ricks G R and R J H Williams 1975 Effects of Atmospheric pollution on Deciduous Wood Land Part 3: Effects on photosynthetic pigments of leaves of *Quercus petraea* (Mattuschka) Liebl. *Environ. Pollut.* **8** 97-106
- Singh M and D N Rao 1982 The influence of ozone and sulphur dioxide on *Cicer arietinum* L.; *J. Indian Bot. Soc.* **61** 51-58
- Syratt W J and P J Wanstall 1969 The effects of sulphur dioxide on epiphytic Bryophytes; *Proc. Eur. Congr. Air Pollut.* **1** 79-85
- Thomas M D 1961 Effects of air pollution on plants; in *Air Pollution* (Monograph WHO) **46** 233-278
- Thomas M D and R H Hendricks 1956 Effects of Air pollution on plants; in *Air Pollution Hand Book* (New York: McGraw Hill Book Company)

Phenology and biochemical changes in male and female shrubs of Jojoba [*Simmondsia chinensis* (Link) Schneider] during different seasons

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Abstract. The phenological and biochemical characteristics of male and female plants of Jojoba were studied during different seasons. The phenological studies indicated that the flowering and fruiting behaviour of this species in the semi-arid conditions of India change considerably with continuous flower formation and two fruiting cycles in a year. Marked differences in the carbohydrates, proteins and nucleic acids were observed between sexes and during different seasons. The pistillate plants had greater metabolic activities with more DNA and RNA than the staminate shrubs.

Keywords. Jojoba; phenology; carbohydrates; amylase proteins; amino acids; DNA; RNA; sex differentiation.

1. Introduction

Sex expression in higher plants is important particularly when the species is dioecious. The role of auxins, gibberellins and kinetins have received considerable attention in the work on sex expression (Heslop-Harrison 1956, 1972; Amruthavalli 1980; Mohan Ram 1980) but very little information is available on the physiological processes and associated biochemical changes in the studies on sex expression and also the effect of environment on such changes (Sivtsev and Sizov 1971; Slonov 1974; Jaiswal and Kumar 1978; Jindal and Singh 1975; Raj Kumar and Gopala Rao 1980a, b; Nazeem and Nair 1981). The dioecious species Jojoba [*Simmondsia chinensis* (Link) Schneider] belonging to the family Buxaceae, has its natural population males out-numbering the females. This species which is receiving attention as a commercial crop has been undergoing domestication in various countries (Prasad 1984). In India the species has been introduced by our Institute and has been grown successfully on coastal sand dunes. Reports are available on early flowering (18 months) and also late flowering after 5 or 7 years (Yermanos 1983; Prasad 1984). Studies conducted on some of the aspects of associated biochemical changes in the male and female plants revealed certain differences. These observations prompted us to study the seasonal variations in the carbohydrates, proteins and nucleic acid metabolisms and phenology in male and female plants of Jojoba.

2. Materials and methods

As Jojoba exhibits heteromorphism in a given population the field study was confined to the bushy type of plants with larger leaf areas [leaf area in males is 6.67 sq cms

± 0.12 and in females $6.76 \text{ sq cms} \pm 0.15$ (leaf area of all the plants were measured by automatic digital Leaf Area Meter, LI-COR, Model LI-3000, USA)].

Leaves of male and female shrubs of Jojoba growing in the sand dunes were collected from the experimental field plots of the Institute. From a large population the plants were marked with metallic tags and each time collections were made only from these plants and the analysis of metabolic constituents were carried out in all the seasons at monthly intervals. Growth characteristics in the staminate and pistillate shrubs during different seasons were recorded.

2.1 Biochemical analysis

Mature leaves collected from the selected male and female plants for the analyses of metabolic constituents *viz* soluble sugars, starch, amylase, proteins, amino acids and nucleic acids.

Nucleic acids and proteins were estimated as described earlier (Prasad and Iyengar 1982). Starch was estimated by the method of McCready *et al* (1950). Soluble sugars and amylolytic activity were estimated by the methods of Yemm and Willis (1954) and Bernfeld (1955). The procedure of Slotta and Primosigh (1951) was used for the determination of amino acids. Proline was estimated by the method of Bates *et al* (1973).

3. Results and discussion

3.1 Phenology

The data on the phenology of Jojoba shrubs growing on the coastal sand dunes of semi-arid region of India are presented in table 1. Field observation revealed that the male population exceeds the female and the ratio of females to males was 47:53. This was comparatively higher than in the xeric conditions of the native Sonoran Desert (20:80 female to male ratio, Gentry 1958). Even in cultivated conditions in California, Yermanos (1983) observed 40:60 ratio of female to male which was far lesser than the sex ratio of Indian coastal climate. This indicates the environmental influence on sex differentiation in Jojoba. Freeman *et al* (1976) investigating on dioecious plants of *Atriplex*, *Distichis*, *Thaliectrum*, *Ephedra* and *Acer* also concluded that for all the species males were abundant in xeric microsites while females over represented in the moisture parts.

The floral bud formation was noticed throughout the year with maximum in monsoon followed by post-monsoon. The rate of formation in both the sexes was retarded in winter. As summer approaches improvement in the floral bud formation was observed in both male and female shrubs. Also the plants showed active growth in all the seasons but less marked in winter. The environmental effects on the plants are fairly well known with the seasonal variations affecting the vegetative growth pattern and flowering. In the shrubs of Jojoba in winter flower budding has not been reported in the native wild stands of Sonoran Desert (Haase 1976; Thomson 1982). But in the Indian coastal climatic conditions the process of flowering occurs throughout the year.

(Dhavanagar).

Monsoon (June–August)	Post-monsoon (September–October)	Winter (Nov–Feb)	Summer (March–May)
Vegetative growth* (peak)	Vegetative growth (moderate)	Vegetative growth (little)	Vegetative growth (moderate)
Flower** budding (profuse)	Flower budding (good)	Flower budding (little)	Flower budding (moderate)
Fruit formation	Fruit maturation	New fruit formation	Fruit maturation

*Vegetative growth in Jojoba during different seasons.

	Monsoon	Post-monsoon	Winter	Summer
Number of lateral branch production	24.00 ± 1.27	15.66 ± 1.66	4.66 ± 0.64	11.00 ± 2.10
Growth increase in height	17.33 ± 2.12	12.33 ± 1.95	5.33 ± 0.76	10.00 ± 2.40

± Standard deviation.

**Number of floral bud formation in staminate and pistillate shrubs of Jojoba during different seasons.

Season	Staminate	Pistillate
Monsoon	50.67	47.00
Post-monsoon	39.67	33.67
Winter	11.67	11.00
Summer	23.00	20.33
C.D. at p 0.05	12.11	9.68
p 0.01	18.34	14.67

photoperiod and low temperature and this may suppress the floral bud formation during winter in the native stands. In its native of Sonoran region (extending in to Mexico and Arizona) the severe cold conditions (less than -5°C) some times lead to frost damage of growing and forcing the plant into dormancy (Yermanos 1983).

Forti (1972) noted that initiation and growth of female flowers are often less conspicuous and last for shorter duration than males although apparent differences in growth behaviour of staminate and pistillate plants were not observed in the mediterranean climate. However, in both the sexes flower formation was initiated after a certain period of vegetative growth, thus indicating the necessity of optimal vegetative growth before induction of floral buds. In the present study vegetative growth was seen

the species tending to behave photoperiodically indifferent.

Further two fruiting cycles were noticed consistently. In the first cycle fruit initiation was observed in June–July and fruit maturation in September to October. In the second cycle fruit initiation began during December to February and maturation of fruits occurred during summer. In the wild populations of Jojoba only one fruiting period in a year has been reported (Haase 1976; NRC 1977). Yermanos (1983) noted only one fruiting period under cultivated conditions in California but in the present study two fruiting periods in a year are noticed consistently which ultimately result in higher yield of Jojoba nuts per bush.

3.2 Carbohydrates and associated metabolism

The differential accumulation of starch examined in male and female shrubs during different seasons is presented in table 2. Maximum levels in monsoon, particularly in July with some decrease in post-monsoon were recorded. The minimum content was noticed in winter in both the sexes. However, gradual increase was observed in summer months. The staminate shrubs showed higher levels than pistillate shrubs in all the seasons with monsoon and winter recording highest and lowest differences respectively

Table 2. Carbohydrates and amylolytic activity in staminate and pistillate shrubs of Jojoba during different seasons.

Season	Starch content (mg/g fresh material)		Soluble carbohydrates (mg/g fresh material)		Amylolytic activity (mg maltose released/g f wt/5 min)	
	Staminate	Pistillate	Staminate	Pistillate	Staminate	Pistillate
Monsoon						
June	11.34 ± 0.40	9.12 ± 0.05	16.14 ± 0.54	20.16 ± 0.87	28.90 ± 1.36	37.60 ± 1.21
July	15.92 ± 0.59	13.08 ± 0.51	13.90 ± 0.66	16.88 ± 0.74	24.50 ± 0.71	32.38 ± 1.24
August	12.78 ± 0.56 (13.21)*	9.46 ± 0.69 (10.55)	17.50 ± 0.79 (15.85)	22.32 ± 0.56 (19.79)	32.68 ± 1.26 (28.69)	41.90 ± 0.99 (37.29)
Post-monsoon						
September	10.78 ± 0.37	9.34 ± 1.01	17.00 ± 0.10	20.00 ± 0.44	26.85 ± 0.55	36.80 ± 0.57
October	11.46 ± 0.63 (11.12)	10.00 ± 0.47 (10.55)	15.20 ± 1.02 (16.10)	20.00 ± 0.32 (20.00)	26.27 ± 1.54 (26.56)	33.00 ± 0.47 (34.90)
Winter						
November	6.22 ± 0.84	5.58 ± 0.27	12.32 ± 0.78	15.20 ± 0.67	21.50 ± 0.71	25.44 ± 1.21
December	9.38 ± 0.29	7.10 ± 0.52	10.00 ± 0.56	12.46 ± 0.52	17.18 ± 1.03	25.00 ± 0.94
January	9.30 ± 0.33	8.16 ± 0.55	10.67 ± 0.32	12.00 ± 0.18	18.32 ± 1.56	22.46 ± 1.20
February	8.46 ± 0.22 (8.34)	6.78 ± 0.37 (6.91)	11.50 ± 0.27 (11.13)	13.40 ± 0.30 (13.27)	21.24 ± 0.58 (19.56)	27.00 ± 0.47 (24.98)
Summer						
March	11.76 ± 0.58	8.64 ± 0.30	15.74 ± 0.46	21.36 ± 0.24	24.50 ± 0.71	31.42 ± 0.76
April	10.86 ± 0.40	7.78 ± 0.37	14.30 ± 0.53	17.80 ± 0.82	26.96 ± 0.92	35.80 ± 1.04
May	12.10 ± 0.42 (11.57)	9.44 ± 0.21 (8.62)	12.85 ± 0.28 (14.30)	15.16 ± 0.36 (18.11)	23.12 ± 0.89 (24.86)	30.24 ± 2.29 (32.49)

± Standard deviation

*Mean values of the season

staminate and pistillate shrubs of Jojoba was probably due to differences in the amylolytic activity. Similar results have been reported for male and female flowers of castor (Gopala Rao and Sastry 1971; Raj Kumar and Gopala Rao 1980a).

The soluble carbohydrates (table 2) showed minimum levels in winter (December and January) while in monsoon and post-monsoon there was maximum accumulation. Higher levels were associated with profuse flower budding (table 1). The pistillate plants had much soluble carbohydrates than staminate shrubs. The optimum periods for soluble carbohydrate accumulation i.e. monsoon and post-monsoon also showed maximum differences between male and female shrubs.

Maximum amylolytic activity (table 2) in both the sexes was observed in monsoon and minimum activity in winter with post-monsoon and summer showing intermediate levels. The highest levels of activity was noticed in females than in males in all the seasons as evidenced by more soluble sugars and less starch content in the former than the latter.

The greater levels of soluble carbohydrates in the leaves of female shrubs of Jojoba may be due to the more amylolytic activity in females than in males. Sivtsev and Sizov (1971) reported that in many dioecious species (hemp, asparagus, dock, willow, boxelder and lombardy poplar) female plants surpassed the males in overall accumulation of soluble carbohydrates during various seasons. They pointed out that the feature was found to be stable under changing weather conditions contending that the female plants acquire greater resistance to adverse natural conditions by accumulating more soluble sugars. The present observations indicate that the carbohydrate levels were low in winter in both male and female shrubs of Jojoba probably because of environmental constraints in photosynthetic activity.

3.3 *Proteins and amino acids*

The protein content of male and female shrubs of Jojoba during different seasons is given in the table 3a. Maximum contents were recorded in the monsoon and post-monsoon with highest levels in August. In winter months the protein levels got reduced to lowest in January while it increased gradually in summer in both the sexes. Irrespective of the seasons lower levels of proteins were observed in the leaves of staminate than those of pistillate shrubs and the physiological status of both the sexes were apparently different. Similar conclusions were also drawn by Malaviya (1965) and Slonov (1974) in male and female plants of hemp.

The amino acid composition (table 3b) from male and female shrubs of Jojoba showed a total of 17 free amino acids in both the sexes. The amino acids asparagine, glutamine, aspartic acid, serine, glycine and glutamic acid were more in females while cystine, threonine, alanine, methionine, valine, phenylalanine, proline were higher in male shrubs. The other amino acids lysine, isoleucine and leucine did not show marked difference between sexes. Dzharidze and Mikeldze (1969) reported greater values for aspartic acid, threonine and tyrosine which are characteristic feature of male plants of *Morus*, *Pistacia*, *Humulus*, *Dioscorea*, *Diospyros*, *Rhamnus*, *Bryonia*, *Ailanthus* and *Populus* while cystine was predominant in females. But in Jojoba the adult plants show higher levels of cystine in males while aspartic acid was more in females. However, the amides both asparagine and glutamine were more in female shrubs while the aromatic amino acids as tyrosine and phenylalanine were more in males. Contrary to this Raj

different seasons.

Season	Staminate	Pistillate
Monsoon		
June	87.00 ± 1.47	98.00 ± 1.78
July	90.84 ± 1.54	105.46 ± 0.72
August	98.60 ± 1.80 (92.15)*	114.62 ± 1.28 (106.03)
Post-monsoon		
September	91.48 ± 1.66	103.20 ± 1.20
October	86.80 ± 2.96 (89.14)	98.15 ± 1.18 (100.68)
Winter		
November	72.46 ± 1.15	81.25 ± 1.29
December	71.40 ± 1.53	79.00 ± 1.34
January	66.70 ± 1.68	73.50 ± 0.89
February	73.14 ± 1.43 (70.91)	85.66 ± 1.25 (79.85)
Summer		
March	79.90 ± 1.81	91.84 ± 0.86
April	90.72 ± 2.22	100.82 ± 1.02
May	84.00 ± 0.89 (84.89)	93.26 ± 1.47 (95.31)

± Standard deviation.

* Mean values of the season.

monsoon.

Amino acid	Male	Female
Cystine	31.38 ± 0.79	26.35 ± 1.58
Lysine	53.80 ± 0.46	54.17 ± 0.53
Asparagine	28.10 ± 1.10	39.47 ± 0.84
Glutamine	80.64 ± 0.65	92.26 ± 0.37
Aspartic acid	86.14 ± 1.28	98.75 ± 1.16
Serine	33.61 ± 1.76	40.26 ± 1.84
Glycine	72.93 ± 0.83	82.42 ± 0.69
Glutamic acid	45.65 ± 0.92	56.52 ± 0.88
Threonine	201.30 ± 1.26	193.64 ± 0.99
Alanine	50.97 ± 2.06	39.90 ± 2.54
Methionine	84.12 ± 0.98	77.50 ± 1.08
Valine	65.14 ± 1.10	59.22 ± 1.24
Tyrosine	29.66 ± 1.03	20.90 ± 0.79
Phenylalanine	134.98 ± 2.10	118.45 ± 2.36
Isoleucine	39.58 ± 0.58	40.30 ± 0.46
Leucine	49.82 ± 1.02	48.80 ± 0.96
Proline	39.18 ± 1.56	30.95 ± 1.08

± Standard deviation.

Kumar and Gopala Rao (1980b) reported that the amide asparagine was high in male flowers of castor while the other amide glutamine was high in female flowers.

3.4 Nucleic acids

The nucleic acid content estimated in the leaves of staminate and pistillate shrubs during different seasons is presented in table 4. DNA content was more in pistillate plants than in staminate in all the seasons. Maximum content was observed in monsoon in both the sexes in synchrony with profuse vegetative growth and flower bud formation (table 1). Significantly less accumulation of DNA during post-monsoon in both the sexes was observed with correspondingly less profuse vegetative and reproductive growth (table 1). In winter the vegetative growth was less with low floral bud formation showing sharp fall in DNA content in both the sexes. In summer there was improvement in the growth of the plants and floral bud formation concomitantly showing considerable increase in DNA content. Thus increase could be seen in the DNA content and flower bud formation which in turn depends on the season when the environmental conditions are favourable.

The RNA content was much higher than DNA and it almost showed similar trend as that of DNA. The female plants during all the seasons had higher RNA contents than the staminate plants.

In the earlier studies higher quantities of protein are reported in female plants than in males and it is contended that a direct proximal relationship possibly existed between

Season	DNA content ($\mu\text{g/g}$ fresh material)		RNA content (mg/g fresh material)	
	Staminate	Pistillate	Staminate	Pistillate
Moonsoon				
June	1273.58 \pm 9.44	1501.71 \pm 5.43	35.14 \pm 1.12	47.98 \pm 1.20
July	1380.29 \pm 10.03	1592.37 \pm 11.48	40.52 \pm 2.43	53.60 \pm 0.66
August	1546.86 \pm 8.79 (1400.24)	1806.56 \pm 33.08 (1633.51)	44.50 \pm 2.10 (40.05)	58.34 \pm 0.32 (53.31)
Post-monsoon				
September	1305.41 \pm 2.55	1650.32 \pm 4.80	39.16 \pm 0.98	50.92 \pm 0.54
October	1182.54 \pm 10.54 (1243.98)	1303.33 \pm 6.28 (1476.83)	37.78 \pm 1.05 (38.47)	48.08 \pm 0.99 (49.50)
Winter				
November	569.00 \pm 3.91	680.00 \pm 4.62	21.12 \pm 0.98	28.85 \pm 1.69
December	704.48 \pm 4.54	847.66 \pm 11.02	26.20 \pm 1.05	30.46 \pm 0.92
January	678.58 \pm 4.42	819.80 \pm 4.72	24.86 \pm 0.34	30.28 \pm 1.09
February	886.60 \pm 5.42 (709.67)	980.24 \pm 3.31 (831.93)	29.40 \pm 0.89 (25.40)	36.12 \pm 0.88 (31.45)
Summer				
March	1083.40 \pm 6.26	1241.24 \pm 2.10	33.04 \pm 0.92	44.36 \pm 1.30
April	1336.35 \pm 3.47	1549.28 \pm 6.26	40.24 \pm 0.70	50.10 \pm 1.59
May	1124.54 \pm 3.91 (1181.43)	1411.00 \pm 7.68 (1400.51)	32.48 \pm 0.75 (35.25)	41.60 \pm 0.44 (45.35)

\pm Standard deviation

*Mean values of the season

the synthesis of RNA, DNA and proteins (Prasad and Iyengar 1982). Contrary to Slonov (1974) the results of the present investigation revealed that during budding and flowering the DNA and RNA levels were invariably higher in females.

Minimum levels of nucleic acids during winter and maximum levels in monsoon in the male and female shrubs of *Jojoba* may be due to the prevailing unfavourable or favourable environmental conditions as also observed for spruce (Sovershaev and Barabin 1972).

The observations on the male and female inflorescences in monoecious plants such as cucurbits (Vlasenko 1969) and corn (Kubarev 1965) showed that the total nucleic acids were reliable criteria for sex determination. In the case of *Jojoba* the DNA levels demarcate the sexes in the adult plants. This clearly signifies the role of DNA in sex expression in this exotic species.

4. Conclusions

The field investigations carried out on bushy type of plants revealed the seasonal variations in metabolic activity between male and female plants. The pistillate plants had higher metabolic activity with more nucleic acids than the staminate. The winter seems to be dormant period of growth for the species with increasing growth and metabolic activities from summer until next winter. Under the semi-arid climate of

female plants of *Jojoba* have higher DNA content and metabolic activities and this may help in differentiation of sex at early seedling stages of the species.

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References

- Amruthavalli S A 1980 Interaction of GA₃ and BA on flowering and sex expression in bulgarian coriander. *Indian J. Plant Physiol.* **23** 14-20
- Bates L S, Waldren R P and Teare I D 1973 Rapid determination of free proline for water stress studies; *Plant Soil* **39** 205-207
- Bernfeld P 1955 Amylases α and β ; *Methods Enzymol.* **1** 149-158
- Dzhaparidze and Mikeladze 1969 Sexual differences in the free amino acid composition of the leaves of dicotyledonous plants; *Fiziol. Rast.* **16** 147-150
- Freeman D C, Klikoff L G and Harper K T 1976 Differential resource utilisation by the sexes of dioecious plants; *Science* **193** 597-599
- Forti M 1972 *Simmondsia* studies in Israel; in *Jojoba and its uses* (eds) E F Haase and W G McGinnis (Arizona: University of Arizona) 13-25
- Gentry H S 1958 The natural history of *Jojoba* (*Simmondsia chinensis*) and its cultural aspects; *Econ. Bot.* **1** 261-295
- Gopala Rao P and Sastry K S 1971 Physiological characterization in male and female flowers in monoecious plant, castor (*Ricinus communis* L.); *Sci. Cult.* **37** 210-211
- Haase E F 1976 Phenology of some native *Jojoba* populations in Arizona, in *La Jojoba Memories De La Conferencia Internacional Sobre* (Mexico: Consejo Nacional de Ciencia y Tecnologia) 39-48
- Heslop-Harrison J 1956 Auxin and sexuality in *Cannabis sativa*; *Physiol. Plant* **9** 588-599
- Heslop-Harrison J 1972 Sexuality of angiosperms; in *Plant Physiology, A treatise* (ed.) F C Steward (New York: Academic Press) C6 133-289
- Jaiswal V S and Kumar A 1978 Flower development and amino acid metabolism in some dioecious plants. *Symp. on advancing frontiers of Plant physiology*, Banaras Hindu University, Varanasi, pp. 6-7
- Jindal K K and Singh R N 1975 Phenolic content in male and female *Carica papaya*. A possible physiological marker for sex identification of vegetative seedlings; *Physiol. Plant* **33** 104-107
- Kubarev P I 1965 Differences between male and female corn inflorescences with respect to nucleic acids. *Fiziol. Rast.* **12** 968-970
- McCready R M, Guggole J, Silveira V and Owens H S 1950 Determination of starch and amylase in vegetables; *Anal. Chem.* **22** 1156-1158
- Malaviya B 1965 On the biochemistry of senescence; in *Growth and Development of Plants* (eds) R D Asan and K K Nanda (New Delhi: Today and Tomorrow's Book Agency) 213-234
- Mohan Ram H Y 1980 Hormones and flower sex; *Plant Biochem. J.* (SM Circar Memorial Volume) 77-8
- Nazeem P A and Nair P C S 1981 Growth and flowering of nutmeg; *Indian Cocoa Arecanus Species J.* **3** 81-8
- NRC 1977 *Jojoba feasibility for cultivation on Indian Reservations in the Sonoran Desert region* (Washington D.C.: National Academy of Sciences) 1-64
- Prasad V V 1984 Physiological aspects of *Jojoba* (*Simmondsia chinensis* [Link] Schneider) Ph.D. thesis, Bhavnagar University, Bhavnagar, India
- Prasad V V and Iyengar E R R 1982 Physiological differences in male and female plants of *Jojoba* (*Simmondsia chinensis* Link); *Curr. Sci.* **51** 1039-1040
- Raj Kumar N and Gopala Rao P 1980a Influence of kinetin and morphactin on changes in sex expression, carbohydrate and nitrogen fractions in castor (*Ricinus communis* L.); *Proc. Indian Acad. Sci. (Plant Sci.)* **8** 457-464
- Raj Kumar N and Gopala Rao P 1980b Amino acids associated with femaleness of castor (*Ricinus communis* L.) induced by kinetin and morphactin; *Proc. Indian Nat. Sci. Acad.* **B46** 191-197

- Sivtsev M V and Sizov S S 1971 Dynamics of sugar accumulation and invertase activity in specimens of different sexes among dioecious plants; *Fiziol. Rast.* **18** 54–59
- Slonov L Kh 1974 Changes of nucleic acid content in leaves of staminate and pistillate hemp plants as a function of nutrition conditions and soil moisture; *Fiziol. Rast.* **21** 864–867
- Slotta K H and Primosigh J 1951 Amino acid composition of crotoxin; *Nature (London)* **168** 696–697
- Sovershaev P F and Barabin A I 1972 Content and localization of nucleic acids in reproductive organs of spruce; *Fiziol. Rast.* **19** 1229–1234
- Thomson P H 1982 *Jojoba hand book* (Bonsall, California: Bonsall Publications).
- Vlasenko V S 1969 The content of nucleic acids in cucumbers of different sexes; *Biol. Zharm.* **22** 38–44
- Yemm E W and Willis A J 1954 The estimation of carbohydrates in plant extracts by anthrone; *Biochem. J.* **57** 508–514
- Yermanos D M 1983 Performance of Jojoba under cultivation between 1973–82. Information developed at the University of California, Riverside, in *Jojoba and its uses through 1982 Proc. 5th Int. Conf.* (ed.) Anna Elias-Cesnik (Arizona: University of Arizona) pp. 197–212

Embryological studies in Gentianaceae

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Abstract. Embryology of *Enicostema hyssopifolium* (Willd.) Verdoorn. and *Exacum saulieri* L. has been investigated. Anther wall development is of the dicotyledonous type and at maturity consists of the epidermis, fibrous endothecium, 1–2 middle layers and the tapetum which is uniseriate with uninucleate cells. Connective tapetum is also differentiated. Pollen grains are triporate and 3-celled at anthesis. Pollen degeneration at various stages of development is common. The bicarpellary, syncarpous and unilocular ovary bears anatropous, unitegmic and tenuinucellate ovules on parietal placentae. Embryo sac development is of the polygonum type. Fertilisation is porogamous and the endosperm is *ab initio* nuclear. Embryogeny is of the Solanad type.

Keywords. Gentianaceae; embryology; *Enicostema hyssopifolium*; *Exacum saulieri*.

1. Introduction

The family Gentianaceae which comprises 80 genera and 900 species is world wide in its distribution. They live in a wide variety of habitats and include arctic and alpine plants, halophytes, saprophytes, hydrophytes and marshy plants. The embryological studies in the family are quite meagre and hence the present investigation is taken up on *Enicostema hyssopifolium* (Willd.) Verdoorn. and *Exacum saulieri* L. The earlier work in the family has been reviewed by Schnarf (1931), Srinivasan (1941), McCoy (1949), Maheswari Devi (1962) and Maheswari Devi and Lakshminarayana (1977).

2. Materials and methods

E. hyssopifolium was collected locally and *E. saulieri* from Kodaikanal and were fixed in formalin-acetic-alcohol. Customary methods of dehydration, infiltration and embedding were followed and the sections were cut between 7–12 μ m in thickness and stained in Delafield's hematoxylin.

3. Observations

3.1 *Microsporangium, microsporogenesis and the male gametophyte*

The androecium usually consists of 4 stamens (figure 2A) in *E. saulieri* and 5 in *E. hyssopifolium*, but rarely 6 stamens as in *E. hyssopifolium* (figure 1A). The archesporium consists of a plate of 4–6 rows of cells (figure 1B) which undergo a periclinal division and produce an outer primary parietal and an inner primary sporogenous layers. The former divides further and give rise the tapetum and 2 wall layers (figures 1 C–E). The hypodermal layer develop fibrous thickenings and forms the fibrous endothecium (figure 2B). Sometimes, it is multiseriate towards the

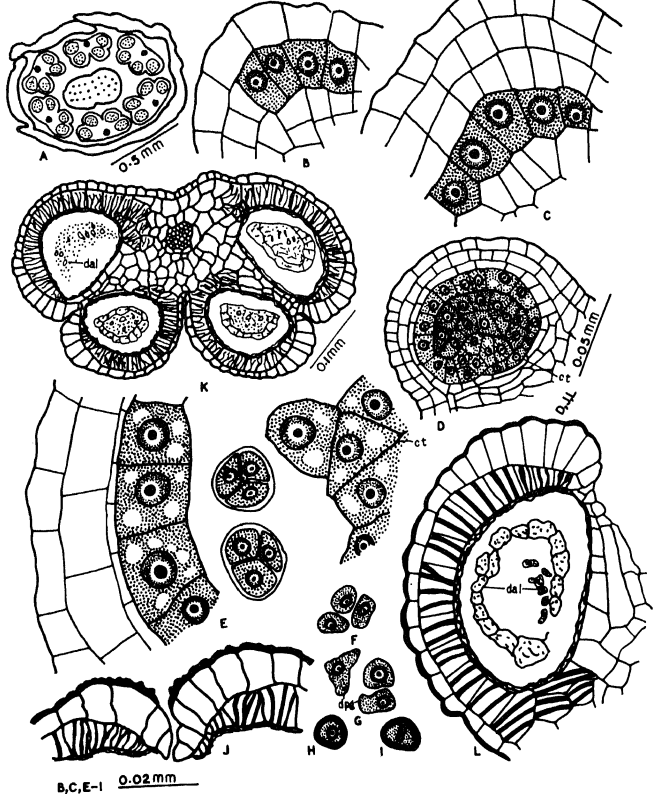


Figure 1. *Encostema hyssopifolium*. A. TS of flower showing 6 tetrasporangiate anthers. B. TS part of anther showing archesporium. C. TS part of anther showing primary sporogenous and primary parietal layers. D. TS anther lobe showing the primary sporogenous cells, tapetum and wall layers. Also note the tapetum from the connective side. E. TS part of anther showing the pollen tetrads, tapetum and wall layers. Note the vacuolated connective tapetum. F. Pollen grains just separated from the tetrad. G. Degenerating pollen grains from a tetrad. H and I. 1 and 2-nucleate pollen grains. K. TS of tetrasporangiate anther showing degenerating pollen grains and fibrous endothecium. Note the fibrous thickenings in the connective cells. L. Sporangium enlarged to show the degenerating pollen grains and the fibrous endothecium.

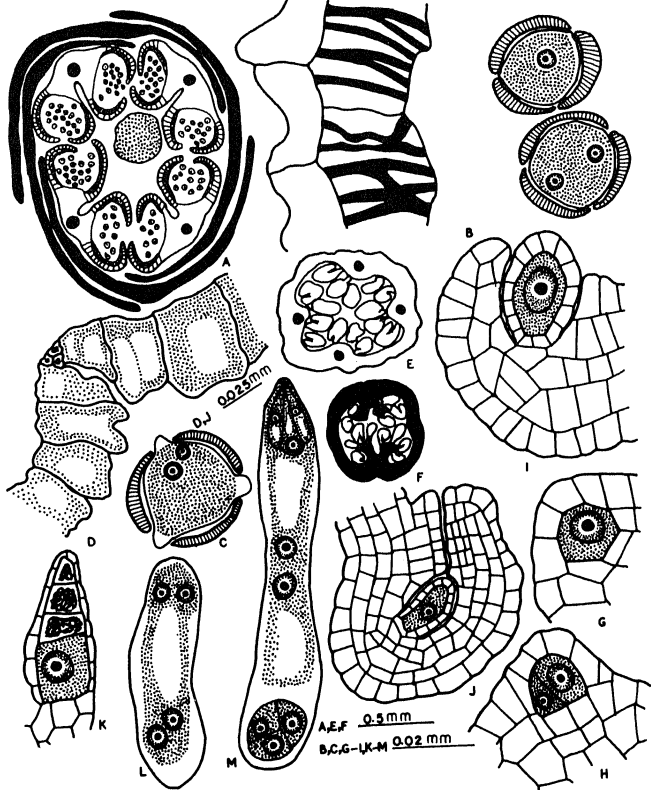


Figure 2. A–E and I and K–M. *Exacum saulieri*. F–H and J. *Enicostema hyssopifolium*. A. TS of flower showing 4 anthers. B. TS part of anther showing pollen grains and fibrous endothecium. C. 2-nucleate pollen grain. D. TS of anther wall showing dehiscing point. E, F. TS of ovaries. G, H. Ovule showing 1 and 2 archesporial cells respectively. I. Ovule showing megaspore mother cell. J. Anatropous, unitegmic and tenuinucellate ovule with megaspore mother cell. K. Megaspore tetrad. L. 4-nucleate embryo sac. M. Mature embryo sac.

connective side (figure 1K). The middle layer is ephemeral. The tapetum is of the secretory type and is uniseriate with uninucleate cells (figures 1D, E). It is dual in origin

in *E. hyssopifolium*. The primary sporogenous cells divide in all planes and forms an extensive sporogenous tissue (figure 1D). Simultaneous cytokinesis in the pollen mother cells result in isobilateral and tetrahedral tetrads of which the latter are more prevalent (figure 1E). The young uninucleate pollen grains possess dense cytoplasm and a large nucleus (figures 1H, 2B) which undergo an asymmetrical division and cuts off a small lenticular generative cell towards the periphery and a large vegetative cell to the centre. In a short time, the wall separating the two nuclei disappear and the generative nucleus moves towards the vegetative nucleus (figures 1I, 2B, C) where it divides to form two male cells. The pollen grains are triporate and 3-celled at shedding. In *E. hyssopifolium* the exine is smooth but in *E. saulieri* (figures 2B, C) it shows reticulate bands of thickenings. The dehiscence is longitudinal and the dehiscing point is clearly demarcated (figures 1J, 2D).

Degeneration of pollen at various stages of development is quite common. The degeneration starts from the tetrad stage and sometimes one, two or all the pollen grains of a tetrad degenerate (figures 1F, G). In *E. hyssopifolium* the entire pollen sac (figure 1L) and in some cases all the 4 pollen sacs of an anther become crumpled and degenerate (figure 1K). In a few instances all the anthers of a flower are degenerated.

3.2 *Megasporangium, megasporogenesis and the female gametophyte*

The ovary is superior, bicarpellary, syncarpous and unilocular with numerous anatropous, unitegmic and tenuinucellate ovules (figures 2I, J) on parietal placentae. The placenta is suppressed in *E. saulieri* (figure 2E) whereas in *E. hyssopifolium* it is forked at the base into two branches (figure 2F). The archesporium is generally unicelled (figure 2G) but rarely 2–3 celled in *E. hyssopifolium* (figure 2H). It directly develops into the megaspore mother cell which undergoes the usual meiotic divisions resulting in a linear tetrad of megaspores (figures 2K, 3A). The chalazal megaspore develops into an 8-nucleate embryo sac of the polygonum type (figures 2K–M, 3A–D). The mature megagametophyte is broader in *E. hyssopifolium* (figure 3D), narrower and elongated in *E. saulieri* (figure 2M). The egg apparatus consists of an egg and two flask shaped synergids which are hooked in *E. hyssopifolium* (figure 3D). The two polars fuse in the centre of the embryo sac. Three antipodals are uninucleate and ephemeral in *E. hyssopifolium* but persist upto the endosperm stage in *E. saulieri* (figure 3F). Rarely 2–3 embryo sacs are observed in *E. hyssopifolium* (figure 3E) and these are probably produced by the functioning of more than one archesporial cell of the ovule.

3.3 *Fertilisation*

Pollen grains are monosiphonous and the fertilisation is porogamous. Pollen tube enters through one of the synergids and discharges its contents through a terminal pore. Syngamy and triple fusion are almost simultaneous and the pollen tube persists upto a few endosperm nuclei are formed (figure 3F).

3.4 *Endosperm*

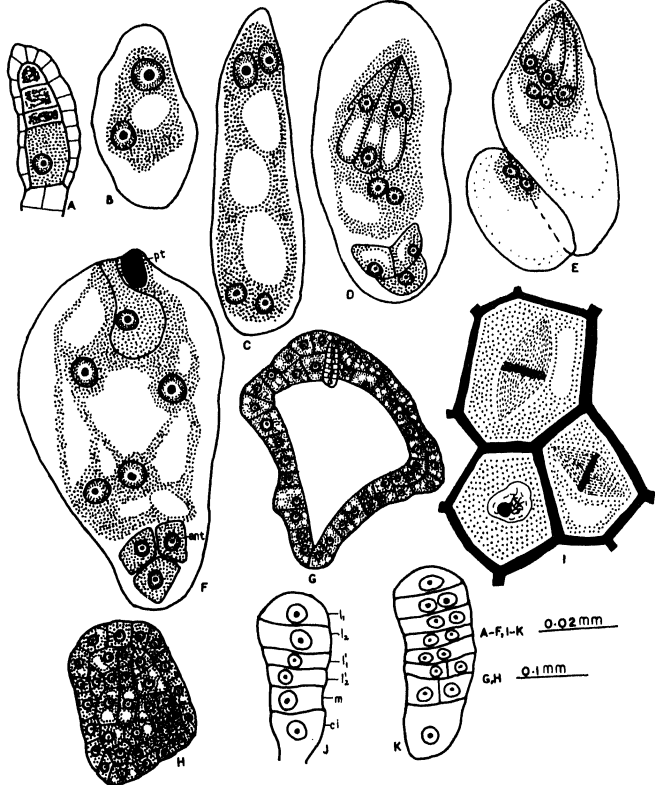


Figure 3. A–E. *Enicostema hyssopifolium*. F–K. *Exacum saulieri*. A. megaspore tetrad. B, C. 2 and 4-nucleate embryo sacs respectively. D. Mature embryo sac. E. Double embryo sacs. F. Embryo sac showing zygote, persistent pollen tube, endosperm nuclei and persistent antipodals. G. Embryo sac showing linear 8-celled proembryo and cellular endosperm. H. Cellular endosperm. I. Nuclear divisions in the endosperm cells. J, K. Embryogeny.

series of simultaneous free nuclear divisions and produce a large number of free nuclei which lie in the periphery of the embryo sac. Cell wall formation commences from the periphery and extends to the centre (figure 3G) ultimately filling the entire embryo sac

3.5 Embryogeny

Due to the paucity of the material, only a few stages in the embryogeny were traced in *E. saulieri*. The zygote divides transversely and produce a two celled proembryo. The terminal cell *ca* and the basal cell *cb* undergo one more transverse division each and give rise to a linear 4-celled proembryo comprising *l*, *l'*, *m* and *ci*. The cells *l* and *l'* undergo one more transverse division each and produce *l*₁, *l*₂, and *l*₁', *l*₂' respectively. Thus a 6-celled proembryo is formed (figure 3J). The lower three tiers namely *l*₁, *l*₂ and *l*₁' undergo further divisions (figure 3K) and contribute to the major portion of the embryo proper. As the derivatives of the *ca* contribute to the embryo proper and *cb* to the suspensor, the embryogeny conforms to the Solanad type.

4. Discussion

In Gentianaceae, the androecium usually consists of 4 or 5 stamens, but 6 stamens in a flower are observed in *E. hyssopifolium*. Fibrous endothecium is present in *E. saulieri* unlike the 3 species of *Exacum* studied by Maheswari Devi (1962). The tapetum is dual in origin and uniseriate with uninucleate cells. Uninucleate tapetal cells were also observed in *Enicostema litterale* (Srinivasan 1941), *Swertia carolinensis* (Mecoy 1949), *Exacum pumilum*, *E. petiolare* and *E. bicolor* (Maheswari Devi 1962) and in *Centaurium ramosissimum* (Vijayaraghavan and Usha Padmanabhan 1969). On the other hand multinucleate tapetal cells were reported in *Limnanthemum cristatum* (Srinivasan 1941; Maheswari Devi 1962) and tapetal cells with nuclear fusions in *L. indicum* (Maheswari Devi 1962). The cells of the connective tapetum are morphologically different from those of the parietal tapetum in the two species studied at present. Such dimorphic tapetum was reported earlier by Periasamy and Swamy (1966) and Vijayaraghavan and Ratnaparkhi (1973). Pollen degenerations as reported in the present investigation, were reported earlier in *E. petiolare*, *Canscora decussata* (Maheswari Devi 1962) and *Centaurium ramosissimum* (Vijayaraghavan and Usha Padmanabhan 1969). The persistent antipodal cells like in *E. saulieri* were reported in *C. ramosissimum* (Vijayaraghavan and Usha Padmanabhan 1969). Embryogeny in *E. saulieri* is of the Solanad type and corroborates with the hitherto investigated taxa.

References

- Maheswari Devi H 1962 Embryological studies in Gentianaceae (Gentianoideae and Menyanthoideae); *Proc. Indian Acad. Sci.* **B56** 195–216
- Maheswari Devi H and Lakshminarayana K 1977 Embryological studies in Gentianaceae; *J. Indian Bot. Soc.* **56** 182–188
- Mecoy R W 1949 On the embryology of *Swertia carolinensis*; *Bull. Torrey Bot. Club* **76** 430–439
- Periasamy K and Swamy B G L 1966 Morphology of anther tapetum of the angiosperms; *Curr. Sci.* **35** 427–430

- Schnarf K 1931 *Vergleichende Embryologie der Angiospermen*, Berlin
- Srinivasan A R 1941 Cytomorphological features of *Limnanthemum cristatum* Griseb. and *Enicostema littorale* Blume; *Proc. Indian Acad. Sci.* **B14** 529-542
- Vijayaraghavan M R and Ratnaparkhi S 1973 Dual origin and dimorphism of anther tapetum in *Alectra thomsonii* Hook, *Ann. Bot.* **37** 355-359
- Vijayaraghavan M R and Usha Padmanabhan S 1969 Morphology and embryology of *Centaurium ramosissimum* Druce. and affinities of the family Gentianaceae; *Beitr. Biol. Pfl.* **45** 15-37

Unusual germination and seedling development in two monocotyledonous dicotyledons

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Abstract. Morphology of germination and seedling development have been studied in *Nymphaea lotus* Auct. var. *pubescens* (Willd) HK. f. and Th. and *Trapa natans* L. var. *bispinosa* (Roxb.) Makino. *Nymphaea* germination has been grouped as a separate type, namely, *Nymphaeal* and that of *Trapa* as *Trapael*. In *Nymphaea* and *Trapa* seedlings, the extension growth is at the mid portion of the cotyledon (mesocotyl) unlike at its base as in the monocotyledons.

Keywords. Germination; seedling development; *Nymphaea lotus* Auct. var. *pubescens* (Willd) HK. f. and Th.; *Trapa natans* L. var. *bispinosa* (Roxb.) Makino; cotyledonary middle piece; mesocotyl; hypocotyl; *Trapa*; *Nymphaea*.

1. Introduction

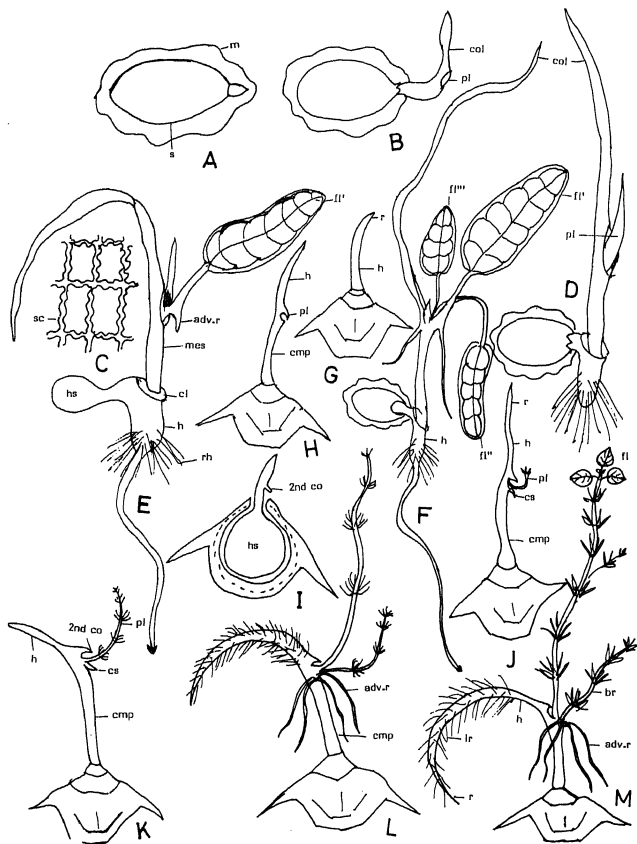
Certain dicots develop only a single cotyledon but the second remains rudimentary or suppressed. The present paper deals with the germination and seedling development in *Nymphaea* (Nymphaeaceae) and *Trapa* (Trapaceae). Hill (1938), Sculthrope (1967) and Cook *et al* (1974), have grouped them under dicots, while Haines and Lye (1975) include them among monocots seedling characters such as presence of coleoptile and mesocotyl. Thus, seedling characters are considered to be of taxonomic importance.

2. Materials and methods

Achenes of *Nymphaea lotus* Auct. var. *pubescens* (Willd) HK. f. and Th. collected from pond grown plants and of *Trapa natans* L. var. *bispinosa* (Roxb.) Makino from Chandola lake 37 km from Ahmedabad were used. Seeds of *Nymphaea* were germinated in Petridishes on moistured filter papers, as well as in a soil in beaker covered with 4" of water. Once germination began, regular observations of seedlings were made under a binocular dissecting microscope and cameralucida sketches drawn. Seedlings were also cleared in KOH for better visualization of the regions like cotyledonary mid-piece and haustorium, mesocotyl, hypocotyl, etc.

3. Results and discussion

Nymphaea has floating leaves on long petioles. The seeds, 1–2 mm long, encapsulated by mucilage swells up considerably soon after imbibition (figure 1A). The seed coat has wavy margined cells (figure 1C). Germination begins 20–25 days or even more after soaking. Plumular sheath (coleoptile) is the first to come out (figure 1B) followed by a



the base of the hypocotyl (figure 1D). Removal of the mucilage and the sheen allows the separation of the haustorium formed by the combined ends of the two cotyledonary lobes ensheathing the plumule—radicle axis.

From the base of the sheathing coleoptile, where there is a plumular depression (figure 1B), the first foliage leaf makes its appearance followed later on by the second and the third (figures 1E, F). The base of the petiole of the first foliage leaf shows an angular protuberance of the first adventitious root (figure 1E). When the coleoptile reaches 2–3 cm in length the radicle also starts elongating. A root pocket protects the radicle at the extreme tip. In the meanwhile, the sheath and haustorial portion of the cotyledon are separated from one another by the development of an elongated cotyledonary middle piece. Haines and Lye (1975) call it the mesocotyl. The radicle and the adventitious roots increase in length and establish the seedling in the ground. The coleoptile by now stops elongation and senesces gradually. The mesocotyl length depends on the depth of sowing.

Trapa, free floating hydrophyte has rosettes of aerial and/or floating leaves and well developed submerged leaves. Fruit development is submerged. In *Trapaceae*, the fruit is a one-seeded drupe is lost its fleshy exocarp leaving a pyrene surrounded by the horned stony endocarp. The fruits of *Trapa* are fixed to the substrate by their own weight and by the spines of the fruit wall. During germination the radicle followed by the hypocotyl emerges from the micropyle, negatively geotropic (figures 1G, H). However, Sculthorpe (1967) does not mention about the radicle at all in *Trapa*.

Of the two cotyledons, one is large and functional which remains within the seed while, the second abortive one is borne aloft on the negatively geotropic hypocotyl (figure 1I). The stalk of the functional cotyledon—the cotyledonary mid-piece—protrudes following the hypocotyl. It bears the cotyledonary sheath at the tip protecting the plumule (figures 1J, K). The plumular bud which is situated in the axil of

Figure 1. A–M. Seed germination and seedling development in *Nymphaea lotus* and *Trapa natans*. **A.** *Nymphaea* seed after imbibition ($\times 20$). **B.** Germinated *Nymphaea* seed showing the emergence of coleoptile and plumule prior to that of radicle ($\times 15$). **C.** A portion of *Nymphaea* seed coat (sc) enlarged to show its ornamentation with wavy margins ($\times 50$). **D.** *Nymphaea* seedling showing the emergence of the plumule from coleoptile and hypocotyl with the radicle at the tip ($\times 15$). **E.** *Nymphaea* seedling cleared in KOH to expose the cotyledonary haustorium within the seed with the seed coat removed. The plumule has emerged through the flaps of cotyledonary lobes, elevated by a mesocotyl. Plenty of fine hairs, rhizoids can be seen around the hypocotylar base ($\times 5$). **F.** *Nymphaea* seedlings showing the emergence of 3 foliage leaves and adventitious roots arising from the tip of mesocotyl ($\times 1$). **G.** *Trapa natans*, initial stages of germination showing a hypocotyl with the radicle at tip ($\times 1$). **H.** *Trapa* seedlings revealing the emergence of cotyledonary middle piece and plumule. Note the negatively geotropic hypocotyl and radicle ($\times 1$). **I.** L.S. of *Trapa* fruit exposing the massive haustorium occupying the entire nut and the second rudimentary cotyledon ($\times 1$). **J and K.** Seedling development of *Trapa* exhibiting the plumule curving towards light ($\times 1$). **L and M.** Advanced stages of seedling development in *Trapa*. Note the curvature of hypocotyl and radicle becoming positively geotropic with many lateral roots developing around ($\times 1$).

Abbreviations: adv. r, adventitious roots; br, branch; cl, cotyledonary lobes; cmp, cotyledonary middle piece; co, cotyledon; col, coleoptile; cs, cotyledonary sheath; fl, foliage leaf; fl', fl'', fl''', 1st, 2nd and 3rd foliage leaves; h, hypocotyl; hs, haustorium; lr, lateral roots; m, mucilage; mes, mesocotyl; pl, plumule; r, radicle; rh, rhizoids; s, seed; sc, seed coat.

later in a rosette shape. Secondary shoot often arises in the axil of the abortive cotyledon. The curvature of the epicotyl towards the water level causes the radicle and hypocotyl to bend in the opposite direction i.e. positively geotropic. Many lateral roots develop around the hypocotyl and the radicle and these, along with numerous adventitious roots developed from the axils of the two cotyledons, help in the establishment of the seedling in the ground (figure 1L).

The massive haustorial part of the cotyledon within the seed (figure 1I) by this time is exhausted of its reserve materials. It never leaves the interior of the nut but gradually decays with it when the seedling establishes completely. Endosperm is absent in *Trapa*.

Monocotyledons possess certain characteristic features, often better developed in the seedling than in the seed before germination, and these features can be used for classification purposes (Haines and Lye 1975). Even though the above authors group *Nymphaea* under hypogeal type of germination, *Nymphaea* combines the characters of epigeal, hypogeal and mesogeal blended in it along with some additional features such as two cotyledonary lobes, reticulate venation of the leaves, presence of a hypocotyl as well as mesocotyl and some unusual behaviour like the emergence of the coleoptile apriori to that of the radicle as reported earlier by Dakshini and Tandon (1970) in a graminaceous member, *Oropetium thomaeum*. All these characters render it necessary to separate *Nymphaea* from the rest and assign it to a separate type namely *Nymphaeal* type.

According to Takhtajan (1969) the only consideration that prevents the *Nymphaeales* being placed in monocotyledons is the supposed "presence of two cotyledons". In both *Nymphaea* as well as *Trapa*, the second cotyledon is present but remains rudimentary and suppressed. *Trapa* exhibits characters of different types blended in it like the presence of a hypocotyl (epigeal), hidden cotyledon, with massive haustorium (hypogeal) and presence of a cotyledonary mid-piece which simulates the mesocotyl of grasses (mesogeal), hence it is termed *Trapael* type.

Burt (1972) reviewed the differences in germination types and concluded that in the monocotyledons extension growth is at the base of the cotyledon, while, in dicotyledons it is primarily in the hypocotyl and the seedling thus has exposed cotyledon and plumule. However, in *Nymphaea* and *Trapa*, the extension growth is chiefly in the mesocotyl and cotyledonary middle piece i.e. at the middle of the cotyledon instead of at the base distinguishing them as intermediary forms. Thus, these two monocotyledonous dicotyledons possess intermediary characters of monocotyledons as well as dicotyledons and stand out unique and deserve special rank.

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References

- Burt B L 1972 Plumular protection and some related aspects of seedling behaviour; *Trans. Bot. Soc. Edinburgh* 41 393-400

- Cook C D K, Gut B J, Rix E M, Schnellur J and Seitz M 1974 *Water plants of the world. A manual for the identification of genera of fresh water macrophytes*. (ed.) W Junk (Hague: B.V. Publishers)
- Dakshini K M M and Tandon R K 1970 An unusual type of germination of graminaceous seed; *Ann. Bot. (London)* **34** 423–425
- Haines W R and Lye K A 1975 Seedlings of Nymphaeaceae; *Bot. J. Linnean Soc.* **70** 255–265
- Hill A W 1938 The monocotyledonous seedlings of certain dicotyledons with special reference to the Gesneriaceae; *Ann. Bot. N.S.* **2** 127–144
- Sculthrope C D 1967 *The Biology of Aquatic Vascular Plants* (London: Edward Arnold Ltd)
- Takhtajan A 1969 *Flowering Plants—Origin and dispersal* (English translation by C Jeffrey) (Edinburgh: Oliver and Boyd)

Reproductive biology of *Torilis arvensis* (Hudson) Link.

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Abstract. *Torilis arvensis* is an andromonoecious umbellifer which produces hermaphrodite and staminate flowers in the ratio of 1:0.36. This figure is recorded for the first time among andromonoecious umbellifers. The species practices geitonogamy on account of weak protandry and little visual impact of its umbels.

Keywords. *Torilis arvensis*; andromonoecy; protandry; geitonogamy.

1. Introduction

Reproduction in plants is accomplished through asexual or sexual processes. The former involves simple replication of the parental genotype and the latter brings forth new diversity in each generation. The main factors involved in producing diverse reproductive units are the breeding and the meiotic system of the species.

Umbellifers exhibit a variety of breeding systems *viz.*, andromonoecy, hermaphroditism, gynodioecy and dioecy. A good deal of literature has accumulated on breeding system of umbellifers (Braak and Kho 1958; Bell 1971; Lloyd 1973; Kumar 1977; Bell and Lindsey 1978; Webb 1979; Lovett Doust 1980; Koul *et al* 1984) but very little work has been published on the meiotic system. The present communication deals with the breeding and meiotic system of *Torilis arvensis*, an andromonoecious umbellifer inhabiting foothills of western Himalayas.

2. Material and methods

All studies were made using plant population growing at Bahu-Wali Rakh, Jammu (India) between January–April, 1984. Individual plants were tagged for studying flowering phenology and pollination mechanisms. The proportion of hermaphrodite and staminate flowers was worked out separately for different umbel orders as well as the whole plant. Observations on anthesis were made on different umbel orders of 5 individuals at different time intervals. Stigma receptivity was detected by calculating pollen load on stigmas of different developmental stages using a malachite green—acid fuchsin stain combination. Pollen counts were made by calculating the number of pollen produced per anther; aniline blue stained preparations were made for the purpose. The figures thus obtained were used for calculating pollen: ovule ratio per hermaphrodite flower, per umbel order and finally per individual (each hermaphrodite flower has 5 anthers and 2 ovules). Fruits set on umbels of different orders were counted, and then the percentage of fruit production was calculated.

dichlorobenzene at $\pm 10^{\circ}\text{C}$ for 3 hr. The pretreated root tips were fixed in 1:3 acetic alcohol for 24 hr. For making chromosome preparations, root tips were hydrolysed at 60°C , in a mixture of 9 parts aceto-orcein and 1 part 1NHCl, and squashed in 1 % aceto-orcein.

For meiotic studies, young inflorescences were fixed in Carnoy's fluid (3 parts absolute alcohol: 1 part glacial acetic acid) for 24 hr and stored in 70 % ethyl alcohol. Buds for appropriate stages were squashed in 1 % aceto-orcein. All studies were made from temporary squashes.

3. Observations

Plants of *T. arvensis* are 20–60 cm tall and are annual. Leaves are 1–2 pinnate, leaf segments are lanceolate and coarsely toothed; the involucrel consists of linear bractlets and the flowers are white. Each population comprised 80–100 plants/m².

3.1 Flowering phenology

The main shoot develops as a leafy stem and terminates in the primary or first order umbel. Lateral shoots are formed within the leaf axils on the main shoot. These terminate in umbels of second order. Further umbels, upto the sixth order arise on lateral branches. The flowering shoot is composed of a sequentially arranged shoots, each terminating in an umbel (figure 1A). The primary umbel is invariably one, but the number of umbels in higher orders varies.

Umbels are concave and compound with 2–4 rays. Each ray terminates in an umbellet which consists of a mixture of hermaphrodite and staminate flowers (figure 1B). The number of umbellets produced by umbels of various orders is 2–3 (2.89 ± 0.11) in primary; 2–4 (2.70 ± 0.61) in secondary; 2–4 (2.87 ± 0.05) in tertiary; 2–4 (2.92 ± 0.08) in quarternary; 2–4 (2.57 ± 0.09) in fifth and 2–3 (2.30 ± 0.13) in sixth order umbels.

The outermost flowers of each umbellet are hermaphrodite and the inner are staminate. The former outnumber the latter in the mature plant (table 1); the ratio ($\text{♀} : \text{♂}$) is 2.79. However, the actual ratios vary among umbels of different orders (table 2) on account of the gradual increase in the number of staminate flowers from primary to higher umbel orders.

3.2 Flower morphology

The hermaphrodite flowers are larger than the staminate; they bear zygomorphic corolla, five stamens and two carpels (figures 2A,D). The anthers are white and basifixed. The ovary is inferior, bilocular, with a single pendulous ovule in each locule. A slightly swollen nectar secreting structure, the stylopodium, caps the ovary and the two styles emerge from within it.

3.3 Anthesis and stigma receptivity

The umbels undergo anthesis in the same sequence in which they differentiate. Primary

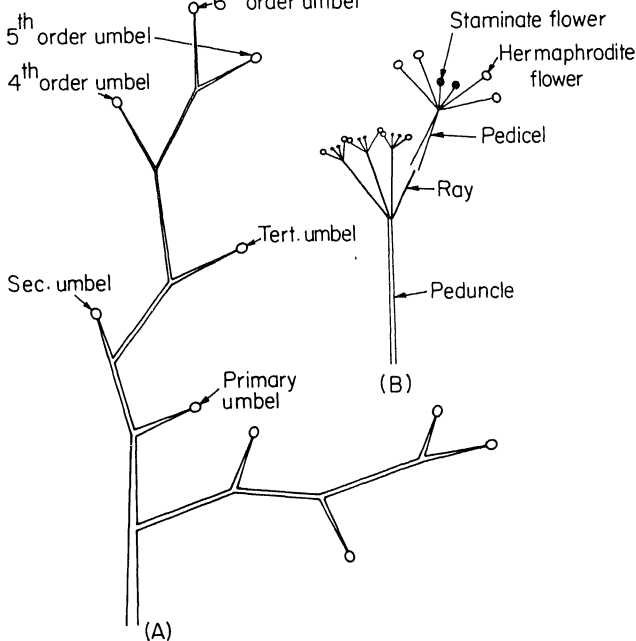


Figure 1. A. Diagrammatic representation of the ordering of umbels of *T. arvensis*. B. A peduncle showing the arrangement of umbellets in an umbel and the flowers within an umbellet.

The peripheral hermaphrodite flowers undergo anthesis prior to the staminate ones. In the hermaphrodite flowers, anthers dehisce before the stigma becomes receptive (figures 2B,C). At the level of the umbel, stigmas of peripheral flowers (which have already shed their pollen), become receptive first followed by those of the central ones. There is sufficient overlap between anthesis of central flowers and stigmatic receptiveness of the peripheral flowers. Similarly, the stigma receptivity and anthesis of other umbel orders overlap (figure 3).

3.4 Pollen: Ovule ratio

On an average, the total number of pollen grains produced per hermaphrodite and staminate flower is 1.633–2.001 (1.807.4 + 76.65) and 1.194–1.398 (1.265.0 + 44.26)

flowers and fruit set in a plant.

Character	Frequency
	Mean \pm S D
Total number of flowers	183.78 \pm 92.98 (56-417)
No. of hermaphrodite flowers	135.36 \pm 71.45 (45-321)
Percentage of hermaphrodite flowers	73.65 \pm 8.72
No. of staminate flowers	48.42 \pm 28.46 (6-122)
Percentage of staminate flowers	26.35 \pm 8.72
Ratio of σ^7 : σ^3 flowers	2.79
Total no. of fruits formed	111.72 \pm 53.71 (36-157)
Percentage fruit set of hermaphrodite flowers	83.43 \pm 0.57

Mean \pm S D of 53 plants

Values in parentheses represent ranges.

respectively. The pollen:ovule ratio per hermaphrodite flower is 903.5 ± 29.15 (797-1,047). Since the proportion of staminate and hermaphrodite flowers vary in umbels of different orders, the pollen-ovule ratios vary (table 3) accordingly. The pollen-ovule ratio for the individual averages 1,129-95.

3.5 Fruit production

Total fruit set of the plant is 83.43%. The fruit output increases from primary to fourth order umbels, and decreases thereafter (table 2).

3.6 Chromosome complement and meiosis

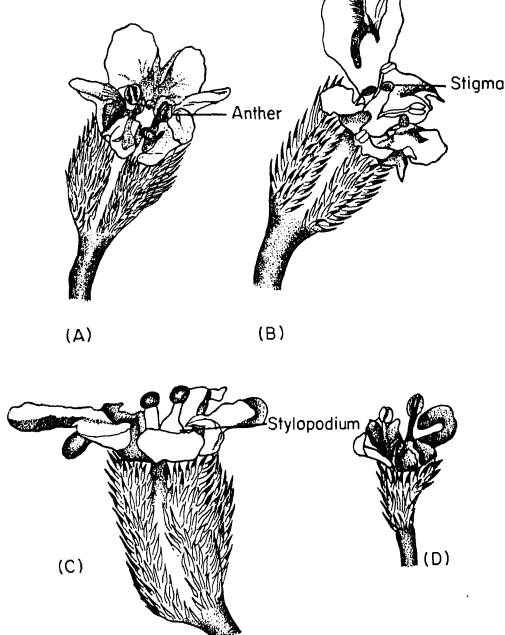
The somatic complement of the species comprises 12 chromosomes measuring 3.39-4.11 μ m (figure 4). On the basis of absolute length and arm ratio, these chromosomes form 6 pairs (figure 5); the first comprises the two longest chromosomes, both having sub-median primary constriction. The second consists of 2 sub-metacentric chromosomes, each having a small satellite at the distal end of the short arm. The third pair comprises 2 sub-metacentric chromosomes with an arm ratio of 2.33. Fourth pair consists of 2 metacentric chromosomes. The fifth consists of 2 chromosomes whose arm ratios approach 1.5. The two smallest chromosomes constituting the sixth pair have their centromere at subterminal position.

The chromosomes pair into 6 bivalents during prophase of meiosis. Each bivalent has 1 or 2 randomly distributed chiasmata at diakinesis (figure 6). The average frequency of chiasmata at metaphase-I (figure 7) is 8.5 per cell and 1.41 per bivalent. Recombination index of the species averages 14.6. Segregation of chromosomes is regular, during anaphase-I as well as II (figure 8). Pollen viability exceeds 76.82%.

Table 2. Quantitative data (mean \pm S D) on staminate and hermaphrodite flowers and fruit set in umbels of different orders.

Umbel order Character	I	II	III	IV	V	VI
No. of umbels studied	53	98	84	42	22	15
Total number of flowers	2015 \pm 4.84 (9-25)	3496 \pm 20.15 (9-90)	3780 \pm 24.79 (7-89)	3528 \pm 19.10 (9-66)	2842 \pm 14.41 (8-53)	2717 \pm 11.2 (10-38)
Number of hermaphrodite flowers	1766 \pm 3.71 (8-23)	2762 \pm 14.38 (6-81)	2820 \pm 15.61 (4-69)	2494 \pm 11.43 (6-46)	1902 \pm 9.93 (5-36)	1792 \pm 8.0 (6-28)
Percentage of hermaphrodite flowers	87.64 \pm 9.85	79.00 \pm 9.74	74.60 \pm 11.05	70.69 \pm 8.21	66.92 \pm 6.86	65.96 \pm 6.4
Number of staminate flowers	249 \pm 1.59 (0-6)	734 \pm 4.29 (0-16)	960 \pm 6.18 (0-24)	1034 \pm 4.98 (3-21)	940 \pm 4.19 (3-17)	925 \pm 3.5 (3-12)
Percentage of staminate flowers	12.36 \pm 9.74	21.00 \pm 9.74	25.40 \pm 11.05	29.31 \pm 8.21	33.08 \pm 6.86	34.04 \pm 6.4
Ratio of hermaphrodite: staminate flowers	1:0.14	1:0.27	1:0.34	1:0.41	1:0.49	1:0.51
Total no. of fruits formed	1020 \pm 5.48 (0-22)	2008 \pm 11.49 (5-54)	2146 \pm 15.11 (3-43)	1808 \pm 11.57 (2-40)	1490 \pm 7.17 (4-23)	1100 \pm 4.2 (3-16)
Percentage fruit set	70.62 \pm 30.77	80.80 \pm 15.60*	86.13 \pm 7.24*	89.30 \pm 10.04*	86.12 \pm 8.29*	83.69 \pm 12.58

Ranges in parentheses. *Significant at 0.001 probability against first order umbel.



Figures 2. A-B. Hermaphrodite flowers at various stages of anthesis ($\times 28$). C. A hermaphrodite flower showing receptive stigma ($\times 28$). D. A staminate flower at anthesis ($\times 30$).

Table 3. Pollen: ovule ratios in different umbel orders.

Umbel order	Pollen grains produced by hermaphrodite flowers	Pollen produced by staminate flowers	Total pollen grain production	Ovules produced	Pollen: ovule ratio
Primary	31,918-68	3,149-85	35,068-53	35-52	987-28
Secondary	49,920-39	9,285-00	59,205-39	55-24	1,071-78
Tertiary	50,968-68	12,144-00	63,112-68	56-40	1,119-02
Quarternary	45,076-56	13,080-10	58,156-66	49-88	1,165-93
Fifth	34,376-75	11,891-00	46,267-75	38-04	1,216-29
Sixth	32,388-61	11,701-25	44,089-86	35-84	1,230-19
Plant	2,44,649-66	61,251-30	3,05,900-96	270-72	1,129-95

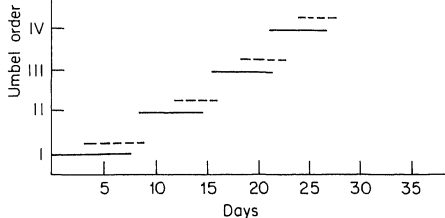


Figure 3. Graphic representation of anthesis and stigma receptivity in various umbel orders.

4. Discussion

Umbellifers exhibit different types of sex expressions, of which andromonoecism is very common. The andromonoecious species vary in the proportion of staminate and hermaphrodite flowers in umbels of different orders. In some taxa (*Daucus carota*, *Anthriscus sylvestris*, *Pastinaca sativa*, *Torilis leptophylla*) there is gradual decrease in the frequency of hermaphrodite flowers from the primary to higher umbel orders; in others (*Thaspium barbinode*, *Zizia* sp.), the primary umbel bears the maximum number of staminate flowers. Although the frequency of staminate flowers increases gradually from primary to higher umbel orders in *Torilis arvensis* as well as *T. leptophylla*, hermaphrodite flowers outnumber the staminate flowers in the former (table 4).

Cruden (1976) reported a uniform ratio of 1 hermaphrodite: 4 staminate flowers at the level of whole plant in 4 umbellifers growing in open habitat. In plants of *Heracleum lanatum* inhabiting a forest site, the production of staminate flowers was considerably higher. This skewness towards the production of more staminate flowers by this species was attributed to its location in the forest where the foraging activity of bees is high which calls for increased pollen production. In *Scandix pecten-veneris* the ratio is 1 hermaphrodite:1.07-1.20 staminate flowers (P Koul, A K Koul and I A Hamal, unpublished results) *T. leptophylla* bearing medium sized umbels falls in between, with the ratio of 1 hermaphrodite:2.73 staminate flowers (Koul *et al* 1984). In an allied species, *T. arvensis*, the staminate flowers are still fewer; the actual ratio between hermaphrodite and staminate flowers being 1:0.36.

Bell and Lindsey (1978) have correlated differences in the ratio of hermaphrodite to staminate flowers with the differences in the degree of dichogamy. The strongly protandrous umbellifers produce more hermaphrodite flowers in the first formed umbels, but in the strongly protogynous taxa the primary umbel has mostly the staminate flowers. In *T. arvensis* there is no difference between the first and the later formed umbels, the hermaphrodite flowers predominate in all. Similar situation



Figures 4–8. 4. A root tip cell showing 12 chromosomes. 5. Karyoidiagram of the cell in figure 4. 6 and 7. Pollen mother cells at diakinesis and metaphase-I. 8. A pollen mother cell at anaphase-I with 6 chromosomes at each pole. (Bar = 10 μ m).

Name of species	I	II	III	IV	V	VI
<i>T. arvensis</i>	87-64	79-00	74-60	70-69	66-92	65-96
<i>T. leptophylla</i> ^a	43-27	28-51	22-17	26-16	—	—

^aFigures from Koul *et al* (1984).

prevails in the weakly dichogamous taxon *Osmorhiza* (C R Bell, personal communication).

In strongly dichogamous umbellifers the staminate phase in all flowers of an umbel is completed before the onset of the pistillate phase (Cruden and Hermann-Parker 1977). Although the flowers in *T. arvensis* are protandrous, there is considerable overlap between the female phase of the peripheral and male phase of the central flowers of the umbel. Obviously, autogamy does not occur on account of dichogamy, conditions for geitonogamy prevail within individual umbels. Production of many hermaphrodite flowers, coupled with very weak dichogamy, increase the chance of geitonogamy in *T. arvensis*.

Although structural features of the flower, existence of unisexual flowers besides the hermaphrodite type, and dichogamy are contrivances for outbreeding plants of *T. arvensis* do not offer much visual attraction to the pollinators on account of small umbel size. Barring some unspecialized ants, which crawl over the umbels, no other visitors were observed on the umbels during the present investigation. Wind also plays a negligible role in the pollination of umbellifers (Proctor and Yeo 1973). Allogamy is therefore, ruled out. Weak dichogamy leading to sufficient overlap between the male and female phases of the flowers of an umbel as well as among the umbels of different orders indicates the operation of geitonogamy. Low pollen: ovule ratio (1,129:95) of the plant is yet another indicator of the geitonogamous nature of the species.

Except for the variability released through the meiotic system, the species does not generate much variability. Low chromosome number and their small size leads to low recombination index. Chiasmata are randomly formed; average chiasmata frequency per cell and per bivalent at metaphase I is 8.5 and 1.41 respectively. Therefore, the individual populations of the species display phenotypic uniformity. Since *T. arvensis* is an annual species, low variability ensures greater stability to its populations.

Acknowledgement

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References

- Bell C R 1971 Breeding systems and floral biology of the Umbelliferae; In *The Biology and Chemistry of Umbelliferae* (ed.) V H Heywood (London: Academic Press) pp 93-108
 Bell C R and Lindsey A H 1978 The umbel as a reproductive unit in the Apiaceae; in *Actes du 2^eeme*

- symposium international sur les ombellifères – Contribution pluridisciplinaires à la systématique*, Perpignan, France, pp 739–747
- Braak J P and Kho Y O 1958 Some observations on the floral biology of the carrot (*Daucus carota* L.); *Euphytica* **7** 131–139
- Cruden R W 1976 Intraspecific variation in pollen-ovule ratios and nectar secretion—preliminary evidence of ecotypic adaptation; *Annals of Missouri Botanical Garden* **63** 277–289
- Cruden R W 1977 Pollen-ovule ratios: a conservative indicator of breeding systems in flowering plants; *Evolution* **31** 32–46
- Cruden R W and Hermann-Parker S M 1977 Temporal dioecism: an alternative to dioecism?; *Evolution* **31** 863–866
- Koul P, Koul A K and Hamal I A 1984 Floral biology of *Torilis leptophylla* (L.) Reichenb. f; *Proc. Indian Acad. Sci. (Plant Sci.)* **93** 449–454
- Kumar C R 1977 Floral biology and breeding system of Bulgarian Coriander (*Coriandrum sativum* L.); *New Botanist* **1–4** 131–135
- Lloyd D G 1973 Sex-ratios in sexually dimorphic umbelliferae; *Heredity* **31** 239–249
- Lovett Doust J 1980 Floral sex-ratios in andromonoecious umbelliferae; *New Phytol.* **85** 265–273
- Proctor M and Yeo P 1973 *The pollination of flowers* (London: Collins and Sons)
- Webb C J 1979 Breeding systems and the evolution of dioecy in New Zealand apoid Umbelliferae; *Evolution* **33** 662–672

Interactions between the root exudates of pearl millet and *Azospirillum brasilense*

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Abstract. Root exudates of different pearl millet varieties showed quantitative differences in organic carbon, reducing sugars, total and amino nitrogen. The growth and nitrogenase activity of *Azospirillum* were stimulated by the addition of root exudates in the culture medium. Root exudates were also found to support the growth of *Azospirillum* in the rhizosphere. Inoculation with *Azospirillum* significantly enhanced the root exudation in axenically grown pearl millet plants accompanied by an increase in the permeability of roots. N_2 -ase activity of the inoculated plants differed among the varieties and was related to the amount of organic carbon released in the exudates. Addition of extraneous carbon source significantly increased the nitrogenase activity of the roots as the carbon compounds provided by the root exudates appear to be inadequate for the optimum expression of nitrogenase activity. The implications of these results in the pearl millet-*Azospirillum* association were discussed.

Keywords. Pearl millet; root exudates; *Azospirillum* inoculation; nitrogenase activity.

1. Introduction

The associative symbiotic bacterium *Azospirillum* has been receiving increasing attention in view of its nitrogen fixing association with the roots of a number of cereal plants (Dobereiner and Day 1975; Subba Rao 1980; Kapulnik *et al* 1981). This organism has been reported to increase the plant growth and yields through a number of processes like nitrogen fixation (Von Bulow and Dobereiner 1975; Kapulnik *et al* 1981), phytohormone synthesis (Tien *et al* 1979; Venkateswarlu and Rao 1983) and increasing the nutrient uptake (Barea *et al* 1983; Lin *et al* 1983). However the various physiological interactions between the host plant and the bacterium leading to the formation of the symbiosis are not yet fully understood. For example, not much information is available on the interactions between root exudates and the microsymbiont although the activity of the bacterium in the root zone is greatly influenced by these compounds as a source of energy and nutrients (Boddey and Dobereiner 1982). The present investigation therefore was conducted to study the interactions between root exudates of pearl millet and *Azospirillum*, such as the biochemical characterization of the root exudates, effect of root exudates on growth and nitrogenase activity of the bacterium, and the influence of inoculation on the exudation pattern and permeability of the roots.

2. Materials and methods

2.1 Collection and purification of root exudates

Four varieties of pearl millet (BJ-104, MP-15, MHB-110 and MHB-118) were grown axenically in glass tubes of 200 × 25 mm size containing acid washed and sterilized quartz sand. Surface sterilized seeds were pre-germinated on plain agar plates and 4 seedlings each were transferred into the tubes which were then kept in an artificially illuminated growth room (14 hr photo period with 30°C day and 25°C night temperatures, light intensity, 12,000 lux). The plants were maintained with Hoagland's half strength mineral nutrient solution containing 50 ppm nitrogen as ammonium nitrate. The moisture level in the tubes was maintained in such a way as to provide adequate water to the plants without causing any water-logging condition around the roots. Plants grew well and a normal root system developed.

Root exudates were collected by removing 12 day old plants from the tubes and rinsing the roots and sand from each tube separately in distilled water. The exudates from all plants of one variety were pooled and centrifuged to remove the suspended matter. The supernatant was concentrated by vacuum evaporation to 1/3 of the original volume and desalted by passing through ion exchange columns (Hussain and McKeen 1963; Rai and Strobel 1966). The crude exudates were passed through Dowex-50 (H⁺ form) and Dowex-1 (formate form) columns to separate it into cationic, anionic and neutral fractions. The individual fractions were evaporated to dryness at 60°C and dissolved in distilled water. For quantitative and thin-layer chromatography (TLC) analysis the different fractions were used directly i.e. cationic for amino acids, anionic for organic acids and neutral for sugars. Alternately all the fractions were pooled together, concentrated and adjusted to a final volume of 1.0 ml representing 10 seedlings. This exudate solution was used to study the effects on the bacterial growth etc.

To study the effect of inoculation with *A. brasilense* on the pattern of exudation in pearl millet (var. BJ-104) 1.0 ml cell suspension of 4 day old *A. brasilense* strain isolated from the roots of pearl millet was added to each tube when the plants were 3 days old. The control tubes received the same amount of autoclaved cells. Root exudates at 8, 12 and 15 days after planting were collected from each set and purified as above for biochemical analysis.

2.2 Biochemical analysis of the exudates

The organic carbon and total nitrogen contents from the crude exudates were estimated by Walkley and Black rapid titration and micro kjeldahl methods respectively (Jackson 1958). The reducing sugars and amino nitrogen were estimated from the respective fractions by Nelson's arsenomolybdate method (Nelson 1944) and ninhydrin method of Moore and Stein (1948) respectively.

prepared initially and appropriate volumes of exudates and sterile distilled water added in each tube to keep the final volume constant. Inoculation was done with 0.1 ml pure washed cell suspension (OD 0.4) of 4 day old *A. brasilense* grown in nutrient broth. The tubes were incubated at 30°C for 72 hr and the optical density was recorded at 520 nm in a Systronics Spectrophotometer. For studying the nitrogenase activity the organism was grown in 7 ml test tubes containing 3 ml of semi-solid malate medium with 50 % of the malic acid (2.5 g/l). The nitrogenase activity was assayed after 48 hr of incubation by acetylene-reduction method as described earlier (Venkateswarlu and Rao 1983).

2.4 Permeability changes in the pearl millet roots

Plants (var. BJ-104) were grown in test tubes containing pure sand as described earlier. One set of tubes were inoculated on the third day with 1.0 ml cell suspension, while the control tubes received the same amount of autoclaved cell suspension. After 15 days the plants were removed by emptying the tubes and the root system was slowly and carefully separated from the sand and gently washed with distilled water. Three replicate samples of 0.5 g fresh roots each from inoculated and control sets were weighed and wrapped in a cheese cloth. These were placed in a conical flask containing 40 ml distilled water. The contents were shaken on a rotary shaker for 5 min and kept at room temperature. The conductivity of the bathing solution was measured in a conductivity bridge at every 1 hr interval.

Alternately root material from control plants was impregnated with pure cell suspension of *A. brasilense* under a brief vacuum to adsorb the bacterial cells on to the roots, and then incubated in a sterile petri dish for 15 min. The roots were rinsed with distilled water and the conductivity of the bathing solution was measured as above.

2.5 Nitrogenase activity in pearl millet-Azospirillum association

Pearl millet plants (BJ-104, MP-15, MHB-110 and MHB-118) were grown in 200 × 25 mm tubes containing equal volumes of sterilized quartz sand and vermiculite as the growth medium and watered with Hoagland's N-free half strength nutrient solution. When the seedlings were 3 day old 1.0 ml pure cell suspension (OD = 0.38) of 4 day old *A. brasilense* and 4 ml of nutrient solution containing 1 % sucrose were added to one set of tubes while the tubes in the second set received the inoculum and 4 ml of nutrient solution. The nitrogenase activity (C_2H_2 -reduction) of intact plants was estimated after 3 weeks without pre-incubation. Acetylene was injected into the tubes directly through an air tight rubber cap and ethylene produced after 24 hr was estimated by gas chromatography (Venkateswarlu and Rao 1983). Appropriate control tubes were included in the assay; uninoculated tubes having plants did not show any acetylene reduction but those inoculated with *A. brasilense* without plants did show some activity which was accounted in the calculations.

2.6 Counts of Azospirillum in the root zone

Pearl millet (var. BJ-104) plants were grown in 200 × 25 mm glass tubes containing sand + vermiculite mixture (1 : 1) and maintained with Hoagland's half strength N-free

washed cell suspension of 4 day old *A. brasiliense* (10^8 cells/ml) was added to all the tubes. Immediately after addition and every 3 days thereafter the numbers of *Azospirillum* in the tubes were estimated by dilution plate count (by transferring whole contents of the tube into the diluent flask) using malate agar medium supplemented with 500 ppm $(\text{NH}_4)_2 \text{SO}_4$.

The data were statistically analysed for analysis of variance. In case of the root exudate data (table 2) as the number of observations were few, the differences between intervals were tested by Kruskal-Wallis one way analysis of variance (H) test.

3. Results

There were marked quantitative differences in the biochemical composition of the root exudates of 4 varieties (figure 1). The exudates of BJ-104 contained the highest amounts of organic carbon and reducing sugars followed by MHB-110, MP-15 and MHB-118.

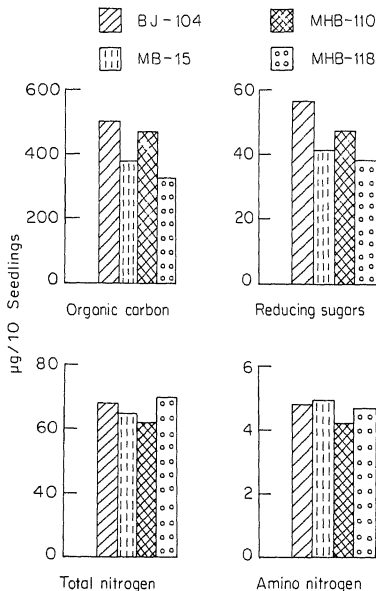


Figure 1. Biochemical composition of the root exudates of pearl millet varieties.

relationship between the seed size of the plant dry weight with the quantity of the root exudates. Organic carbon in the exudates varied from 1.6–2.1 % of the plant dry weight in different varieties and C:N ratio from 4.7 to 7.5. Reducing sugars in the exudates were much higher than amino nitrogen. Qualitative analysis of the exudates on TLC showed the presence of amino acids like glutamic acid, tryptophane, cysteine and asparagine besides two unidentified spots. Sucrose, glucose, fructose and xylose were among the sugars identified. Three organic acids viz. citrate, malate and succinate were also detected but in extremely small quantities.

Root exudates when incorporated in the culture medium had a stimulatory effect on the growth of *A. brasilense* which varied with the variety and the maximum was observed with BJ-104 (table 1). There was a gradual increase in the growth with increasing concentration. The exudates from BJ-104 which contained highest amounts of organic carbon and reducing sugars also showed maximum stimulatory effect.

The nitrogenase activity followed a similar trend as that of growth (table 1). With different varieties the increase in the activity with 20 % exudates varied from 12–25 % over control. The pellicle formation was much quicker and dense in the semi-solid medium at higher concentrations.

The exudation of organic carbon, reducing sugars, total and amino nitrogen increased gradually with increasing age of the plant under axenic conditions (table 2). Further inoculation significantly enhanced the exudation of all these compounds and the effect became more pronounced at the later stages. The increase varied from 16.7–19.9 % in case of organic carbon and 15.2–17.3 % in case of reducing sugars from 8th–12th day. The data on organic carbon was consistent with a *t* value significant at 5 % for inoculation while the Kruskal-Wallis *H* for intervals was not significant. Although there was an increase in the other 3 components it was not statistically significant whereas the Kruskal-Wallis *H* for periods was significant at 5 % level. Increased root exudation upon inoculation has been suggested to be due to several reasons including changes in the permeability of the root cells and altering the metabolism of the roots. In the present study inoculated roots showed higher cell

Table 1. Effect of root exudates of pearl millet on the growth and nitrogenase activity of *A. brasilense*.

Conc. of exudates (%)	Growth (optical density at 520 nm)					Nitrogenase activity ($\mu\text{mol C}_2\text{H}_4 \text{ tube}^{-1} \text{ h}^{-1}$)				
	BJ-104	MP-15	MHB-110	MHB-118	Mean	BJ-104	MP-15	MHB-110	MHB-118	Mean
Control			0.202					214		
5	0.218	0.209	0.215	0.210	0.213	229	222	226	219	224
10	0.236	0.221	0.231	0.225	0.228	238	226	240	228	233
15	0.259	0.235	0.250	0.242	0.246	246	231	245	239	240
20	0.281	0.262	0.273	0.269	0.271	268	241	261	256	256
Mean	0.248	0.231	0.242	0.236	0.239	245	230	243	235	238
SEM \pm for varieties			0.016					2.4		
Concentrations			0.016					2.4		
Interaction			0.033					4.7		

Treatment	8	12	15	Mean	SD	t-value	8	12	15	Mean	SD	t-value
	Organic carbon ^a						Total nitrogen ^a					
Control	412	466	492	456.7	36.6	3.31*	59	67	75	67.00	9.91	2.18
Inoculated	481	548	590	539.7	49.40		68	79	88	78.3	9.99	
Mean	446.5	507.0	541.0	498.2			63.5	73.0	81.5	72.7		
Kruskal-Wallis												
H for periods				5.54						6.34*		
	Reducing sugars ^a						Amino nitrogen ^a					
Control	41	52	66	53	12.07	1.56	3.9	4.8	5.6	4.77	0.97	1.66
Inoculated	58	56	75	63	10.08		4.6	5.9	6.8	5.77	1.12	
Mean	49.5	54.0	70.5	58.0			4.25	5.35	6.20	5.27		
Kruskal-Wallis												
H for periods				7.65*						6.81*		

^a µg/10 seedlings.

* significant at 5% level.

Table 3. Permeability changes in pearl millet roots as influenced by inoculation.

Incubation time	Conductivity (µmhos/cm/g fresh root)	
	Uninoculated	Inoculated
1	310 ± 8.5	334 ± 8.5
2	381 ± 7.1	428 ± 28.3
3	418 ± 8.5	456 ± 11.3

Mean ± Standard deviation.

permeability than those treated with autoclaved cells (table 3). The permeability was higher by 12.3% in the inoculated roots incubated for 2 hr. However when the roots were directly impregnated with cell suspension the change in the permeability was much higher (44.6% over the control after 2 hr of incubation).

Nitrogenase activity of the intact plants inoculated with *A. brasilense* differed significantly among varieties (table 4). The activity varied from 12.5 nmol C₂H₄/hr/plant in MHB-118 to 21 nmol in BJ-104. However, the amount of carbon available in the exudates appear to be inadequate for the optimum activity of the microsymbiont. When extraneous carbon source as sucrose is provided in the root zone there was a spurt in the nitrogenase activity of all the varieties (table 4), accompanied by a marked increase in the numbers of *Azospirillum* in the root zone. The increase in the activity due to carbon supplementation varied from 125–272% in different varieties.

Periodical counts of *Azospirillum* in the root zone of the inoculated plants grown axenically showed that plant roots exert a favourable influence on the microorganism in the root zone. The numbers in the rhizosphere increased rapidly from 11.2 × 10⁷–17.5 × 10⁷ cells/tube from day 0–10, while in the non rhizosphere it declined to

varieties and the influence of carbon supplementation.

Variety	Nitrogenase activity (nmol C ₂ H ₄ /Plant/hr)	
	Without sucrose	With sucrose
BJ-104	21.0 ± 4.2	49.0 ± 4.5 (133.0)
MP-15	14.5 ± 3.1	32.7 ± 6.2 (125.0)
MHB-110	17.5 ± 2.8	45.0 ± 3.9 (157.0)
MHB-118	12.5 ± 3.5	46.5 ± 5.5 (272.0)

Mean ± Standard deviation.

Figures in parentheses indicate % increase over control.

10.2×10^7 during the same period. However from day 10 onwards the numbers declined under both the conditions, but more so in the non rhizosphere.

4. Discussion

In the present study a close relationship between carbon compounds exuded by the roots and the *in vitro* growth of *A. brasilense* in the exudate media as well as the nitrogenase activity of the intact plants was observed. Rovira (1965), Lee and Gaskins (1982) and Beck and Gilmour (1983) have emphasized the role of root exudates in the nutrition and colonization of rhizosphere microflora. The quantitative differences observed in different varieties of pearl millet and its relationship with the nitrogenase activity indicates that differences in root exudates may be one of the major factors responsible for the genetic variation in the root associated nitrogenase activity. Von Bulow and Dobereiner (1975) in maize, Vlassak and Reynders (1978) in wheat and Pohlman and McColl (1982) in barley have emphasized the role of root exudates in the varietal variation of nitrogenase activity. The stimulation of *in vitro* growth and nitrogenase activity by the exudates further indicates the favourable effects on the microsymbiont. In general, the *in vitro* growth of the organism and the nitrogenase activity of the intact plants were proportional to the organic carbon and reducing sugars released in the exudates emphasizing the role of energy supply to the organisms. With *Sorghum-Azospirillum* association Lee and Gaskins (1982) using [¹⁴C] have found that photosynthetically fixed carbon moves quickly into the roots and the growth of bacteria in the rhizosphere is proportional to the release of carbon compounds in the root zone.

Inoculation under axenic conditions has significantly increased the exudation in pearl millet (table 2). Increase in the root exudation in the presence of microorganisms has been reported by Rovira (1965), which was attributed to the possible changes in the permeability of the roots induced by the microbial metabolites. Lee and Gaskins (1982) in *Sorghum* and Beck and Gilmour (1983) in wheat have also found increased exudation in the presence of N₂-fixing bacteria. In the present study the cell permeability of inoculated roots has increased markedly which might have contributed to the enhanced exudation. The permeability change was more marked when the roots were impregnated with the pure cells. Although this treatment can not be compared with the other two it suggests a definite physiological interaction between the bacteria

the culture media (Garcia et al 1978) and it is possible that these cell wall hydrolyzing enzymes may play an important role in increasing the cell permeability.

The population in the root zone increased markedly and remained much higher than in the non-rhizosphere indicating the possible *in vivo* effects of root exudates. However it showed a declining trend after 10 days which might be due to the inadequate supply of the root exudates to the rapidly multiplying bacteria and a possible competition for O_2 between the bacteria and respiring roots in the closed system (Martin and Glatzle 1982).

The nitrogenase activity of the 4 varieties was related to the organic carbon exuded by the roots indicating the role of the exudates as a carbon source to bacteria. However, when external carbon is provided there was a marked increase in the activity and the population in the rhizosphere indicating that the amount of exudates released by the roots may not be adequate for the optimum expression of this association. Hess and Kiefer (1981) and Lethbridge and Davidson (1983) have also reported the presence of considerable latent activity in various grass-diazotrophic associations which was expressed only upon providing extraneous carbon source. However most of these experiments including the present study were conducted under axenic conditions where plants grow with some constraints, whereas the actual quantum of exudates in the soil under natural conditions might be much larger. Further insoluble suspended materials in the root zone have been demonstrated to be equally important in the rhizosphere (Barber and Martin 1976) while most of the studies so far have concentrated on the affects of soluble compounds on the rhizosphere microflora.

Although it is difficult to conclusively evaluate the role of root exudates as a source of energy supply from the present study mainly because of the limitations imposed by investigating small seedlings, the results demonstrate the crucial role of these compounds in the formative stages of the associative symbiosis.

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References

- Barber D A and Martin J K 1976 The release of organic substances by cereal roots into the soil; *New Phytol.* **76** 69-80
- Barea J N, Bonis A F and Olivares J 1983 Interactions between *Azospirillum* and VA-mycorrhiza and their effects on growth and nutrition of maize and rye grass; *Soil Biol. Biochem.* **15** 705-709
- Beck S M and Gilmour C M 1983 Role of wheat root exudates in associative nitrogen fixation; *Soil Biol. Biochem.* **15** 33-38
- Boddey R M and Dobereiner J 1982 Association of *Azospirillum* and other diazotrophs with tropical gramineae. *Transactions; 12th Int. Cong. Soil Sci.*, New Delhi pp 28-47
- Dobereiner J and Day J M 1975 Associative symbiosis in tropical grasses; characterization of microorganisms and nitrogen fixing sites; in *Proceedings of 1st International Symp. on N_2 -fixation* (eds) W E Newton and C J Nyman (Washington State Univ. Press) pp 518-538
- Garcia M U, Hubbell D H and Gaskins M H 1978 Process of infection of *Panicum maximum* by *Spirillum lipoferum*; *Ecol. Bull. (Stockholm)* **26** 373-379

- Hess D and Kiefer S 1981 Induction of bacterial nitrogenase activity in-vitro associations: A comparison of the inducing capabilities of *Triticum aestivum* and *Sorghum nigricans*; *Z. Pflanzenphysiol.-Bd.* **101S** 15-24
- Hussain S S and McKeen W E 1963 Interactions between straw-berry roots and *Rhizoctonia fragariae*; *Phytopathology* **52** 541-545
- Jackson M L 1958 *Soil chemical Analysis*; Printice Hall of India Private Limited, New Delhi
- Kapulnik Y, Kigel J, Okon Y, Nur I and Henis Y 1981 Effect of *Azospirillum* inoculation on some growth parameters and N-content of wheat, sorghum and *panicum*; *Plant Soil* **61** 65-70
- Lee K J and Gaskins M H 1982 Increased root exudation of ^{14}C -compounds by sorghum seedlings inoculated with nitrogen fixing bacteria; *Plant Soil* **69** 391-399
- Lethbridge G and Davidson M S 1983 Root associated nitrogen fixing bacteria and their role in the nitrogen nutrition of wheat estimated by ^{15}N -isotope dilution; *Soil Biol. Biochem.* **15** 365-374
- Lin W, Okon Y and Hardy R W F 1983 Enhanced mineral uptake by *Zea mays* and *Sorghum bicolor* roots inoculated with *Azospirillum brasilense*; *Appl Environ. Microbiol.* **45** 1775-1779
- Martin P and Glatzle A 1982 Mutual influence of *Azospirillum* spp. and grass seedlings; *Experientia Suppl.* **42** 108-210
- Moore S and Stein W H 1948 A modified ninhydrin reagent for the photometric determination of amino acids and related compounds; *J. Biol. Chem.* **186** 367-369
- Nelson N 1944 A photometric adaptation of the Somogyi method for determination of glucose; *J. Bio. Chem.* **153** 375-380
- Pohlman A A and McColl J G 1982 Nitrogen fixation in the rhizosphere and rhizoplane of barley; *Plant Soil* **69** 341-352
- Rai P V and Strobel G A 1966 Chemotaxis of Zoospores of *Aphanomyces Cochlioides* to sugar beet seedlings; *Phytopathology* **56** 1365-1369
- Rovira A D 1965 Plant root exudates in relation to the rhizosphere microflora; in *Ecology of soil borne plant pathogens* (eds) K F Baker and W C Snyder (London: John Murray) pp 170-186
- Subba Rao N S 1980 Crop response to microbial inoculation; in *Recent Advances in Biological Nitrogen Fixation* (ed.) N S Subba Rao (London: Arnold) pp 406-420
- Tien J M, Gaskins M H and Hubbell D H 1979 Plant growth substances produced by *Azospirillum brasilense* and their effect on growth of pearl millet (*Pennisetum americanum* L.); *Appl. Environ. Microbiol.* **37** 1016-1024
- Venkateswarlu B and Rao A V 1983 Response of pearl millet to inoculation with different strains of *Azospirillum brasilense*; *Plant Soil* **74** 379-386
- Vlassak K and Reynders L 1978 Factors affecting biological dinitrogen fixation by associative symbiosis in temperature regions; *Proc. Int. Symp. Use of Isotopes and Radiation in Research on Soil-Plant Relationships* (IAEA Colombo) pp 137-147
- Von Bulow J F W and Dobereiner J 1975 Potential for nitrogen fixation in maize genotypes in Brazil; *Proc. Nat. Acad. Sci. USA* **72** 2389-2393

The veinsheath syndrome in Cunoniaceae. II. The genera *Acsmithia*, *Codia*, *Cunonia*, *Geissois*, *Pullea* and *Weinmannia*

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Abstract. The high order foliar vein sheathing of 40 species representing 6 genera of Cunoniaceae was examined. The type, orientation, distribution, and proportion of the sheathing elements around the veinlet, including the terminal cells, are variable and useful for generic circumscription and subdivision. Terminal cells are tracheoidal or sclereidal types. A general trend towards the formation of specialized sclerenchymatous terminal elements is supported.

Keywords. Veinsheath syndrome; vein termini cells; Cunoniaceae.

1. Introduction

In the preceding paper in this series (Rao and Dickison 1985), the considerable anatomical variation in the type and distribution of sheath cells associated with the ultimate leaf venation in the endemic New Caledonian genus *Pancheria* was described. The present investigation represents a continuation of that study, surveying the varied veinsheath features of the higher order foliar venation in *Acsmithia*, *Codia*, *Cunonia*, *Geissois*, *Pullea* and *Weinmannia*. The objectives of this study are to assist in clarifying the taxonomy, evolution, and generic definitions in this primarily Southern Hemisphere family.

2. Materials and methods

A total of 40 species belonging to 6 genera were investigated in the present study. Materials previously cited by Dickison (1975) were reexamined. Specimens not studied earlier are listed here under their respective genera. Leaves were processed in accordance with the techniques outlined in our earlier paper (Rao and Dickison 1985).

3. Observations

3.1 *Acsmithia* Hoogland

A genus of 14 species of small to medium-sized trees, inhabiting rainforests and also exposed clearings and ridge scrub. The plants are distributed in Fiji, New Caledonia, Australia, New Guinea, and the Moluccas. Additional specimens examined: *Acsmithia densiflora* (Brongn. and Gris) Hoogl., Dickison 220 (NCU); *A. elliptica* (Pampan.)

Hoogl., Dickison 186 (NCU); *A. undulata* (Vieill.) Hoogl., Dickison 262 (NCU).

The vein reticulum is moderately broad and forms prominent areoles of different shapes and sizes. Veinlets are absent or of the branching or nonbranching type, with or without free endings (figures 22–27). Sheath cells around the venation, including free vein endings, show minor differences of diagnostic value and can be used to recognize 3 anatomical categories.

Veinlets, including free vein endings have abundant sheathing composed of parenchymatous cells of variable form. The walls of the sheath cells in juxtaposition with the tracheids are noticeably thicker than those facing the mesophyll. This condition occurs in *A. brongniartiana*, *A. densiflora*, *A. pubescens* and *A. vitiensis* (figures 23, 24, 27). The other minor differences in veinlet sheathing are related to the continuity of varied sheath cells, including those surrounding the vein termini as in *A. pubescens* and *A. vitiensis*, the presence or absence of sclerenchymatous fibres around the veinlets as in *A. brongniartiana* and *A. pubescens*, and also the occurrence of contrasting veinlets with or without sheath cells in different areas of the same lamina, as in *A. densiflora*. The observable differences and variations among species of this anatomical grouping are of potential systematic significance.

Veinlets are ensheathed by globoid or elongate cells that are either thick-walled and pitted or thin-walled with differential wall thickenings. These conditions are illustrated by *A. pedunculata* and *A. reticulata* (figures 22, 26). The veinlets in *A. pedunculata* have more or less elongate sheath cells, whereas sheathing in *A. reticulata* consists of globular cells having differential wall thickenings.

Veinlets are swollen or bulbous, rarely branched, and are either devoid of sheathing or are surrounded by allantoid or irregularly shaped, thick-walled and pitted cells. These features are present in *A. davidsonii*, *A. elliptica*, *A. pulleana* and *A. undulata*. *A. davidsonii* and *A. pulleana* have rather massive veinlets that are heavily ensheathed and often interconnect the main veins surrounding the areoles (figure 25). The veinlets are free from sclerenchyma, unlike the more major veins forming the areolation that possess both sclerenchymatous fibres and associated lignified and pitted sheath cells. *A. undulata* has massive veinlets that extend across the areoles and that are generally devoid of sclerenchyma, but have sheath cells that are more or less globoid in shape and only occasionally sclerified. Along the major veins the sheath cells are elongated, pitted, and heavily sclerified. *A. elliptica* is characterized by vein reticula, including free vein endings, that are ensheathed with thick-walled, pitted, lignified, sclereid-like cells. The free vein endings are generally clavate and have sheath cells that are globoid or of no definite form.

3.2 *Codia J. R. and G. Forster*

An endemic New Caledonian genus comprised of about 11 species of shrubs and treelets, ranging from humid mountain forests to dry, scrubby “maquis” vegetation. Additional species examined: *C. incrassata* Pampan., Dickison 289 (NCU).

The vein reticulum is moderately broad and elaborate with well-developed areolation of triangular or quadrangular outline. Veinlets are prominent, mostly nonbranching and with free endings. The sheath system along the venation bordering the areoles is either absent or sparse, and not conspicuous. Sheathing is prominent, however, around the veinlets including free vein endings.

the presence of specialized tubular cells of irregular width that often extend beyond the vein terminations. These terminal cells are thin or thick-walled, devoid of wall sculpture, distributed at various levels along the veinlets, and occur as single elements or in clusters. They are weakly birefringent and positive to the phloroglucinol-HCL test. The orientation and extension of these distinctive cells beyond the vein endings suggests that they are constituents of the sheathing system. The extended, terminal cells show variation with respect to general topography. In *C. incrassata*, *C. nitida* and *C. obcordata* (figures 16–18) they are oriented mostly parallel to the leaf surface, whereas in *C. albicans*, *C. albifrons*, *C. discolor* and *C. montana* (figures 19–21) the majority of vein endings possess elongated, tubular cells disposed more or less at right angles to the leaf surface, thereby giving a spheroidal or subspheroidal appearance in the cleared laminae. The presence of these specialized terminal cells and their extension into the areoles is a characteristic feature of *Codia*.

3.3 *Cunonia* Linnaeus

A genus of about 17 species of small to medium-sized trees, occurring in rainforests or exposed ridges at lower to middle elevations, often growing along streams or in drainage areas. With the exception of one species (*C. capensis* from South Africa) all species are restricted to New Caledonia. Additional specimens examined: *C. atrorubens* Schlechter, Dickison 233 (NCU); *C. austrocaledonica* Brongn. and Gris, Dickison 260 (NCU); *C. balansae* Brongn. and Gris, Dickison 230 (NCU); *C. pterophylla* Schlechter, Dickison 238 (NCU); *C. purpurea* Brongn. and Gris, Dickison 257 (NCU).

The vein reticulum is moderately prominent with imperfect areolation of irregular shape and size. Veinlets are either branching or nonbranching with tapering or club-shaped terminations. The sheathing cells surrounding the areoles are mostly elongated or globular but in the veinlets sheathing is either absent or consists of a few enlarged cells. Terminal tracheoidal cells occur as solitary elements or in clusters, and sometimes differentiate into sclereids. On the basis of veinlet anatomy including the vein endings, the investigated species can be separated into 3 general groups.

Veinlets simple or branched, linear or curved, biseriate, with sparse sheathing composed of thin-walled cells of no definite form. In *C. atrorubens* (figure 3) and *C. macrophylla*, a few spheroidal to subspheroidal sheath cells occur that are terminal or subterminal in position. These cells have thick walls, wide lumina, irregular pitting, and are birefringent when viewed under polarized light. The major vein reticula in these species are accompanied by sparsely distributed, inconspicuous sheath cells and a thick strand of sclerenchyma.

Veinlets are simple or branched, linear or curved, and are surrounded by clusters of thin-walled, misshapen cells that increase in frequency from the veinlet base to apex, resulting in a cluster of irregularly arranged cells at the termini of the free vein endings. This condition is present in *C. austrocaledonica* (figure 2) and *C. deplanchei* (figures 1, 34). Most of the sheathing cells are thin-walled; however, in *C. deplanchei* a few sheath cells are occasionally sclerified with pitted walls. These spheroidal sclereids are restricted to the sides of areoles adjacent to the midrib region of the lamina.

Veinlets are simple or branched, linear or curved, and characteristically bulbous or club-shaped with a single layered or multiple layered sheath. The sheath cells around

characterize *C. balansae*, *C. bullata* (figures 5, 35), *C. latifolia* (figure 6), *C. montana* (figure 4), *C. pterophylla* (figure 7), and *C. purpurea* (figure 8). Vein reticula have inconspicuous sheaths in all the above species except *C. pterophylla*, in which a uniseriate sheath of distinctly spheroidal cells is present (figure 7). A striking feature in *C. bullata* is the presence of subspheroidal, diffuse sclereids along the sides or near the vein reticula, especially toward the abaxial surface of the lamina (figure 5).

3.4 *Geissois Labillardiere*

A genus of about 18 species of medium-small to medium-large trees, occurring in open scrub or rainforest, with a distribution in Fiji, New Caledonia, Solomon Islands, and eastern Australia.

The characteristic vein reticulum of *Geissois* is moderately broad with a well-developed areolation pattern composed of meshes of relatively consistent size and shape. The sclerenchymatous elements bordering the areoles are conspicuous, whereas the veinlets possess multiseriate sheath cells. The ensheathed veinlets are with or without free vein endings and often have papillate outgrowths. Veinlet sheathing cells are sometimes extended and connect with the sheath cells of the major veins. Although there is much intergradation among species in veinlet structure, the range of variation in veinlet anatomy can be summarized as follows: veinlets with multilayered sheaths and with bulbous or swollen head endings, as in *G. intermedia* (figure 29); veinlets with or without free endings and with short, oblong, nonsclerenchymatous sheath cells, illustrated by *G. superba* (figure 31); veinlets with more or less rounded, non-sclerenchymatous sheath cells, as in *G. imthurnii*; veinlets often showing nipple-like outgrowths and sheathed with short, oblong and thin-walled cells, a few terminal or subterminal elements having thick walls, as in *G. ternata*; veinlets mostly devoid of free vein endings and possessing a dense sheathing of sclerenchymatous fibres that are continuous with the major veins, as in *G. benthamiana* (figure 30); and veinlets free from sclerenchyma whereas fibres surround the major veins, as in *G. hirsuta* (figure 28).

3.5 *Pullea Schlechter*

A genus of 3 species of small to medium-sized trees of rainforests, occurring in Fiji, Queensland, Moluccas, and New Guinea.

The vein reticulum is moderately broad with areolation of varied shape and size. The veinlets range between unbranched and threesome branched, and the vein endings have enlarged terminal tracheoidal elements. Sheathing around the areoles and veinlets is not conspicuous, and is composed mostly of narrow, elongated cells that sometimes have nipple-like protrusions and sclerified, pitted, secondary walls.

The principal point of interest in all three species of *Pullea*, namely *P. glabra*, *P. stutzeri* and *P. versteeghii*, is the presence of large, thin-walled, more or less spheroidal or slightly lobed terminal cells that form components of the sheath system of the veinlets (figures 32, 33). As observed by Dickison (1975), the terminal elements have sparse differential wall thickenings, except in the region of contact with other cells.

high elevation rain forests, widely ranging throughout the tropics including Central America northward to Mexico, West Indies, Malaysia, Melanesia, and Polynesia, as well as temperate New Zealand and Chile. The genus is absent in India, Australia, and mainland Africa.

The vein reticulum is moderately conspicuous with distinct areolation of varied shape and size. The veinlets are often 2 or 3 times branched with bulbous or specialized cells at the free vein terminations. The areoles of all species are bordered by a dense sclerenchymatous sheath. Sheath cells along the veinlets are generally prominent, especially at the vein endings. The terminal sheath cells are tracheoids, that are sometimes differentiated into sclereids.

The investigated species possess rather distinct features of diagnostic value at the anatomical group level.

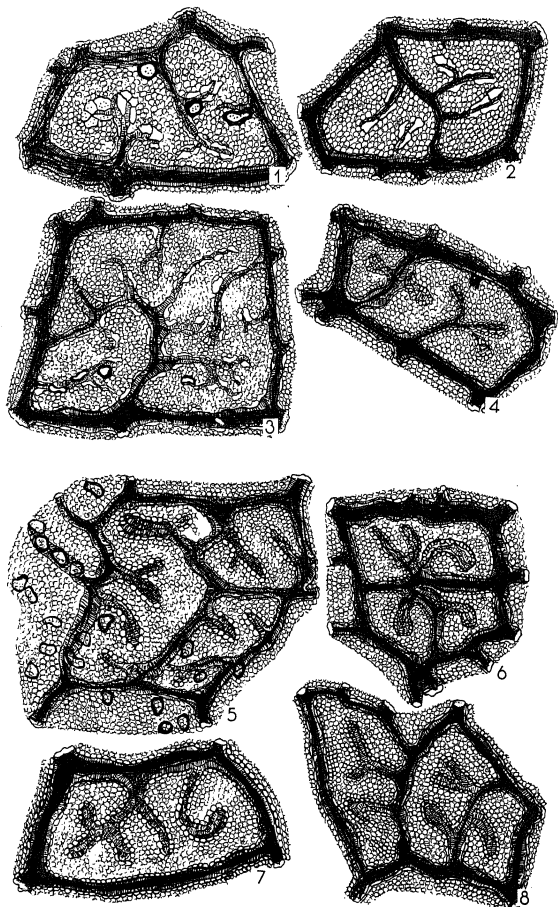
Veinlets straight or curved, biseriate, simple or branched, with clusters of relatively small-sized, thin-walled, globose cells at the free vein endings. The sheathing around the veinlets and the vein reticula is multilayered and composed of relatively thick-walled, sclerotic cells. These features are present in *W. agaroides*, *W. pinnata*, *W. pubescens*, and *W. tomentosa* (figures 9–11).

Veinlets are straight or curved, single or branched, and generally devoid of sheathing, but the vein endings occasionally possess enlarged, helically thickened sclerotracheoids, as well as a few thick-walled, lignified and pitted sclereids of varied shape and size. Similar sclereid types may also be scattered along the veinlets and vein reticula. Both sclereid and sclerotracheoid idioblasts are birefringent, and their wall reaction is positive to the phloroglucinol-HCL test. These features occur in *W. affinis*, *W. luzoniensis*, *W. negrosensis* and *W. richii* (figures 12–15, 40–43).

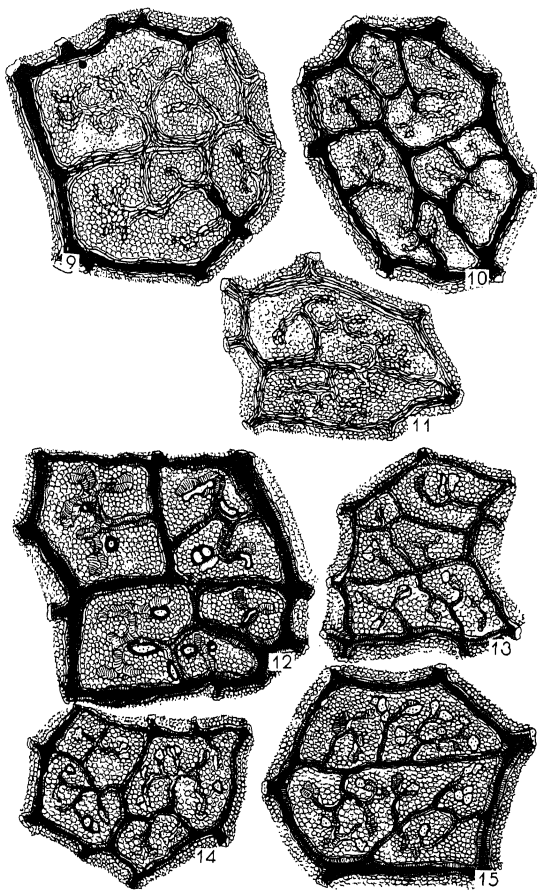
Veinlets are straight or curved, simple or branched, and club or bulbous shaped with conspicuous multilayered sheaths. The sclerotic sheath cells vary in size and shape, but are characterized by having thin-walls adjacent to the tracheids and thick-walls elsewhere. These features occur in *W. brachystachya*, *W. caripensis*, *W. cochensis*, *W. crenata*, *W. silvatica*, and *W. subsessiliflora* (figures 36–39, 44, 45). *W. trichosperma*, however, can be distinguished by the presence of sclereid-like cells near the vicinity of the midrib and the dissimilarity of the vein reticula and veinlets to other species of *Weinmannia*.

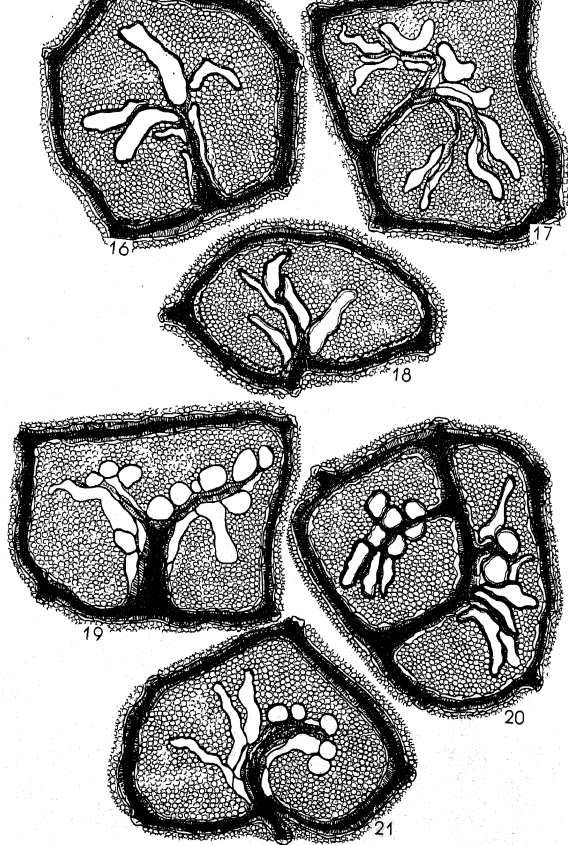
4. Discussion

The present study, as well as an earlier contribution dealing with the genus *Pancheria* (Rao and Dickison 1985), confirm the observations of Dickison (1975) that genera of Cunoniaceae are quite variable in the type of sheathing associated with mature high order foliar venation and in the nature of the cells at the free terminations of the fine veins. This diversity is further evidence of the wide variation in morphology and anatomy of the leaves of this family, and is an apparent reflection, in part, of the extremes of habitats that these plants occupy. In general, the occurrence of sheath cells bordering the vein reticulum, and also especially the veinlets and vein endings, are widely distributed in dicotyledons as-a-whole. Sheathing cells occur in many taxa of flowering plants belonging to such diversified taxonomic and ecologic groups as

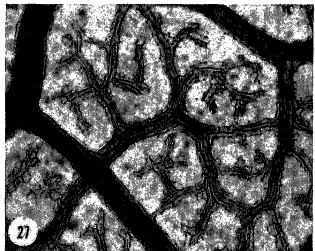
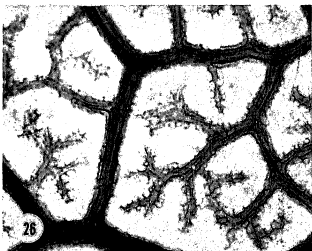
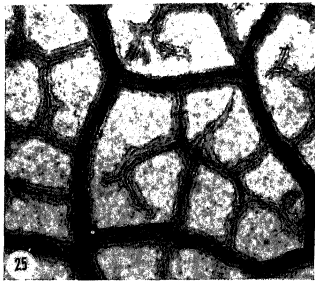
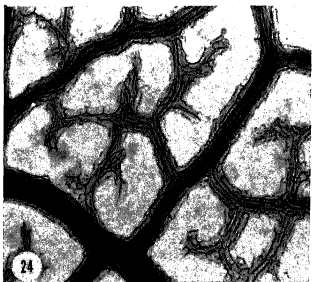
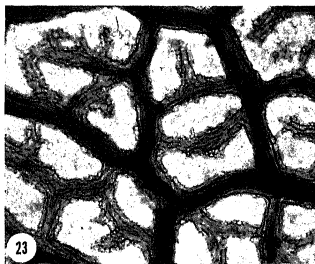
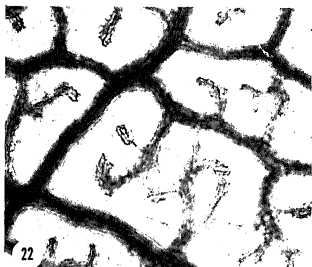


Figures 1-8. Camera lucida drawings of cleared leaves of *Curatella* showing veins.



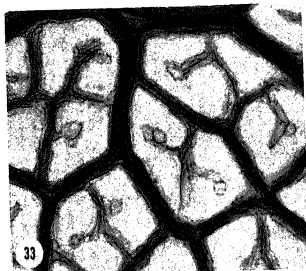
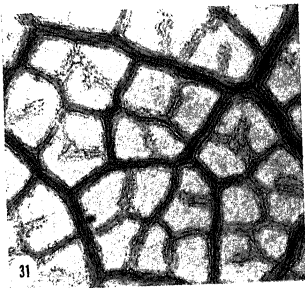
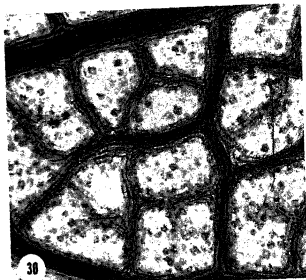
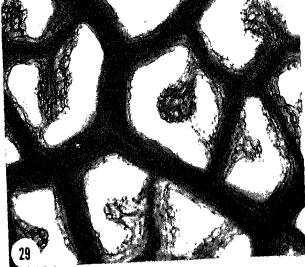
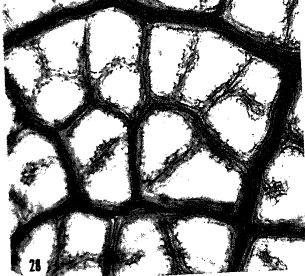


Figures 16-21. Camera lucida drawings of cleared leaves of *Codia* showing veinlet sheathing. All $\times 100$. 16. *C. incrassata*—(Dickison 289). 17. *C. nitida*—(Guillaumin and Baumann-Bodenheim 12149). 18. *C. obcordata*—(Baumann-Bodenheim 14145). 19. *C. discolor*—(Guillaumin and Baumann-Bodenheim 6682). 20. *C. montana*—(Hurlimann 1765). 21. *C. albicans*—(Guillaumin and Baumann-Bodenheim 12363).

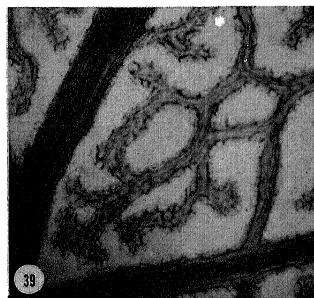
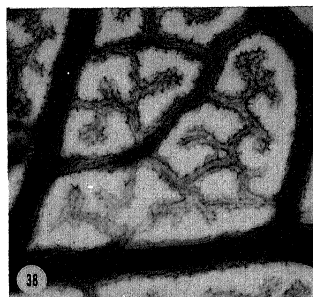
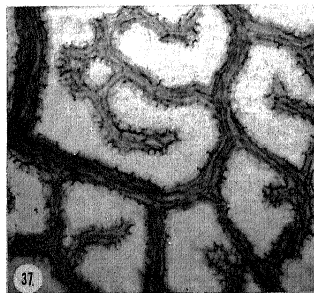
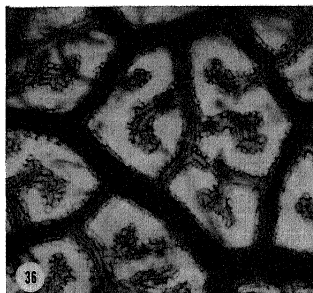
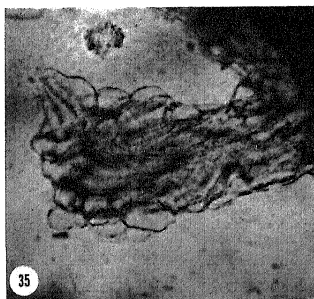
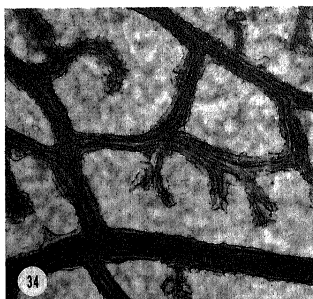


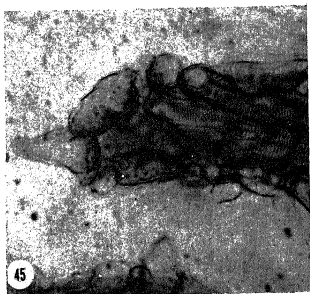
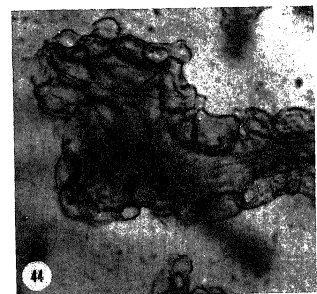
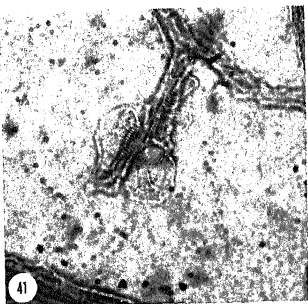
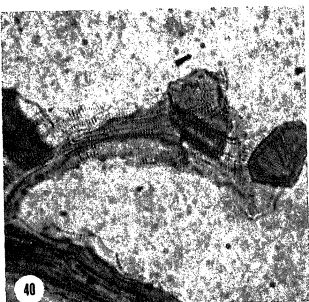
Figures 22–27. Cleared leaves of *Acsmithia* showing veinlet sheathing. All $\times 100$. 22. *A. pedunculata* — (Franc 674). 23. *A. pubescens* — (Guillaumin 8410). 24. *A. vitiensis* — (Parham 1910). 25. *A. davidsonii* — (White 10706). 26. *A. reticulata* — (Brass 12693). 27. *A. brongniartiana* — (MacKee 4432).

Magnoliaceae (Tucker 1964), Dilleniaceae (Dickson 1969, 1970; Rury and Dickson 1977), Euphorbiaceae (Seheal and Paliwal 1974), Ericaceae (Lems 1964; Rao and



Figures 28-33. Cleared leaves of Cunoniaceae showing veinlet sheathing. All $\times 100$. 28. *Geissois hirsuta*—(White 2158). 29. *G. intermedia*—(Buchholz 1548). 30. *G. benthamiana*—(US #1507847). 31. *G. superba*—(Smith 8772). 32. *Pullea stutzeri*—(White 10598). 33. *P. glabra*—(Clemens 11108).





Figures 40-45. Cleared leaves of *Weinmannia* showing veinlet sheathing. All $\times 400$. 40. W.

The presence or absence, and orientation of sheath cells around the areoles and veinlets in the Cunoniaceae taxa studied reveal distinct patterns. Sheathing may either be absent around the ultimate venation, or, similar to dissimilar parenchymatous or sclerenchymatous sheath cells are present and range between sparsely and abundantly distributed. Although the importance of sheath cells to normal physiological activity is not totally clear, sheathing cells can be compared to some extent with cells of the endodermis. For example, the walls of the sheath cells in a few species of *Cunonia* and *Weinmannia* show structural similarity to endodermal cells, especially in their differential wall thickening.

In the Cunoniaceae, veinlets are either conventional in structure or possess sheath cells in groups of 2 or 3 at the endings. The terminal cells are tracheoidal or sclereidial forms that are comparable with those described in other families of angiosperms (Rao 1980; Rao and Bhattacharya 1978; Rao and Das 1978, 1979a, b, c; Rao and Chakraborti 1985; Rao and Jacques-Felix 1978; Lersten and Carvey 1974). Although there is considerable variation in the structure of terminal cells, the coexistence of diverse types of terminal elements appears to be constant at specific levels in some taxa (Foster 1946; Rao 1957).

The present study has also confirmed that the syndromes of veinsheath features in some genera of Cunoniaceae are more or less distinct, and can be utilized for generic circumscription and subdivision. The minor differences in sheath anatomy, however, cannot generally be used for distinguishing cunoniaceous taxa at the specific level. Taxa of *Acsmithia* can be grouped on the basis of their possessing parenchymatous or sclerenchymatous sheath cells around the ultimate veins. The endemic New Caledonian genus *Codia* is readily identifiable by the occurrence of diagnostic, extended tubular terminal cells that are oriented either parallel or at right angles to leaf surfaces. *Geissos* is distinguished by the sheathed veinlets, in which free vein endings are characteristically absent or sparse, and that are associated with a consistent, well-developed pattern of areolation, and *Pullea* has large terminal or subterminal, thin-walled cells at the vein endings. *Cunonia* includes one group of species with more or less linear veinlets surrounded by sparse sheathing and associated with terminal or subterminal sclereids, a second group of species in which sheathing is relatively abundant with sheath cells increasing in frequency from the base to apex of the veinlet, and a third group of species characterized by club-shaped or bulbous veinlets. In *Weinmannia* groups of species can be reorganized anatomically by possessing either abundant sclerotic sheath cells around the vein reticula and clusters of globose cells at the vein termini, or sparsely sheathed reticula with veinlets having enlarged helical tracheoids in juxtaposition with sclereids of varied shapes, or bulbous or club-shaped veinlets with abundant sheathing.

In view of the apparent close relationship between *Cunonia* and *Weinmannia*, and even the suggestion that they are congeneric, it is interesting to note the strong similarities in high order venation and sheathing between these taxa, including the presence of terminal sclereids.

Dickson (1975) was of the opinion that there existed in the Cunoniaceae a general trend toward the formation of specialized terminal cells, particularly one resulting in sclerenchymatous terminal elements. The coexistence of varied vein terminal cells in a few taxa of Cunoniaceae not only supports this generalization but also confirms an earlier view expressed by Rao and Mody (1961) that genera having markedly enlarged

diversity in terminal veinlet elements remains obscure. Furthermore, it is very apparent that the formation of well-developed areolation of constant size and placement in which free veinlet endings are absent, or short and unbranched, represents a structurally more advanced condition.

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References

- Arbo M M 1977 Venacion foliar menor en *Byttneria* Loebl. (Sterculiaceae); *Bonplandia* (Corrientes) 3 211–268
- Banerji M L and Das S 1972 Minor venation pattern of Indian species of *Acer*; in *Advances in Pl. Morphol.* (eds) Y S Murty, B M Johri, H Y Mohan Ram and T M Varghese (Meerut: Sarita Prakashan) pp. 51–57
- Banerji M L and Sirkar G 1974 Minor venation pattern of Indian species of *Cassia*; *Bull. Bot. Soc. Bengal* 26 1–5
- Dede R A 1962 Foliar venation patterns in the Rutaceae; *Am. J. Bot.* 49 490–497
- Dickson W C 1969 Comparative morphological studies in Dilleniaceae IV Anatomy of the node and vascularisation of the leaf; *J. Arnold Arbor.* 50 384–410
- Dickson W C 1970 Comparative morphological studies in Dilleniaceae V Leaf anatomy; *J. Arnold Arbor.* 51 89–101
- Dickson W C 1975 Leaf anatomy of Cunoniaceae; *Bot. J. Linn. Soc.* 71 69–76
- Foster A S 1946 Comparative morphology of the foliar sclereids in the genus *Mouriria* Aubl.; *J. Arnold Arbor.* 27 253–271
- Inamdar J A and Murthy G S R 1981 Vein ending in some Solanaceae; *Proc. Indian Acad. Sci. (Plant Sci.)* 90 53–58
- Jain D K 1978 Studies in Bignoniaceae III Leaf architecture; *J. Indian Bot. Soc.* 57 369–386
- Lersten N R and Carvey K A 1974 Leaf anatomy of ocotillo (*Fouquieria splendens*; Fouquieriaceae), especially vein endings and associated veinlet elements; *Can. J. Bot.* 52 2017–2021
- Lems K 1964 Evolutionary studies in the Ericaceae. II. Leaf anatomy as a phylogenetic index in the Andromedeae; *Bot. Gaz.* 125 178–186
- Rao T A 1957 Comparative morphology and ontogeny of foliar sclereids in seed plants 1. *Memecylon* L., *Phytomorphology* 7 306–330
- Rao T A 1980 Aspects and prospects of foliar sclereids in Angiosperms, in *Current trends in botanical research*, (eds) M Nagaraj and G R Mallik (New Delhi: Kalyani Publishers) pp. 67–72
- Rao T A and Bhattacharya J 1978 A review on foliar sclereids in angiosperms; *Bot. Surv. India* 20 91–99
- Rao T A and Chakraborti S 1985 The veinlet syndrome in the tribe Andromedeae (Ericaceae); *Proc. Indian Acad. Sci. (Plant Sci.)* 94 639–654
- Rao T A and Das S 1978 Idioblasts typology in the taxonomy of *Capparis*; *Curr. Sci.* 47 917–919
- Rao T A and Das S 1979a Comparative typology and taxonomic value of foliar sclereids in *Hibbertia* Andr.; *Proc. Indian Acad. Sci.* B88 161–174
- Rao T A and Das S 1979b Typology of tracheoids in angiosperms; *Proc. Indian Acad. Sci.* B88 331–345
- Rao T A and Das S 1979c Leaf sclereids-occurrence and distribution in the angiosperms; *Bot. Not.* 132 319–324
- Rao T A and Dickson W C 1985 The veinsheath syndrome in Cunoniaceae 1. *Pancheria*; *Proc. Indian Acad. Sci. (Plant Sci.)* 95 87–94
- Rao T A and Jacques-Felix H 1978 Les types de sclerites foliaires et la classification des *Memecylon* africains; *Adansonia* 18 59–66

- Rao T A and Mody K J 1961 On terminal sclereids and tracheoid idioblasts; *Proc. Indian Acad. Sci.* **B53** 257-262
- Rury P M and Dickison W C 1977 Leaf venation patterns of the genus *Hibbertia* (Dilleniaceae); *J. Arnold Arbor.* **58** 209-241
- Schadel W E and Dickison W C 1979 Leaf anatomy and venation patterns of the Styracaceae; *J. Arnold Arbor.* **60** 8-27
- Sehgal L and Paliwal G S 1974 Studies on the leaf anatomy of *Euphorbia* II. Venation pattern; *J. Linn. Soc. Bot.* **68** 173-208
- Tucker S 1964 The terminal idioblasts in magnoliaceous leaves; *Am. J. Bot.* **51** 1051-1062
- Verghese T M 1969 A contribution on the foliar venation of Scrophulariaceae, in *Recent advances in the anatomy of Tropical seed plants* (ed) K. A Choudhury (Delhi: Hindustan Publishing Corporation) pp. 253-263

Fungi of wastewaters and stabilisation pond

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Abstract. The fungal composition of domestic wastes and waste stabilisation pond water samples from two geographically distinct localities—Dharwad and Gulbarga in Karnataka State was studied. Fungi of wastewaters and stabilisation pond at Dharwad comprised of 13 species belonging to 8 genera and 16 species belonging to 11 genera respectively and that of wastewaters at Gulbarga comprised of 19 species belonging to 12 genera. Species belonging to 9 genera were recorded commonly in both the localities. Statistical analysis of the results indicated that *Aspergillus flavus*, *Aspergillus niger*, *Penicillium oxalicum* and *Trichosporon* sp. occurred in all the samples with high percentage of occurrence and high degree of consistency. Occurrence of some of the organisms commonly in two different environments (from the point of organic loading)—wastewaters and stabilisation pond—reflects upon their lymaphilic nature.

Keywords. Fungi; wastewaters; stabilisation pond.

1. Introduction

The bacterial composition of faeces, domestic wastes and stabilisation ponds is well understood (Geldreich 1978). Yet little information is available on their fungal composition. Pioneering studies on these lines were carried out by Cooke and his coworker (Cooke 1954 a, b, c; Cooke and Matsuura 1966; 1969; Cooke 1970; 1977). However information on fungi of wastewaters and stabilisation ponds in India appears to be rather limited excepting for a few reports (Vittal Rao *et al* 1965; Rai 1981; Vittal and Aravazhi 1981). The present report aims to shed some more light on the fungal composition of domestic wastewaters and stabilisation ponds in India.

2. Materials and methods

Two cities Dharwad and Gulbarga in the northern part of Karnataka State differing in geographical as well as climatic conditions were selected. Dharwad lies between latitude 14°78' to 15°50'N and longitude 74°48' to 76°00'E and has a comparatively cooler climatic conditions, with an average temperature of 28.8°C (maximum in summer months being 36°C) and an annual rainfall of 148.34 cm. Gulbarga on the other hand occupies latitude 16°12' to 17°46'N and longitude 76°04' to 77°42'E, has a warmer climate recording temperatures of 44–46°C in May and has an annual rainfall of

in the Botanical Gardens. Its design and operational characteristics are thus: 45.75 m length, 13.70 m breadth and 1.22 m depth, surface area of 627 m², inflow of wastewater 77282 L/day, holding capacity of 764630 L and a detention period of 10 days (Rodgi and Kanbur 1971). Water samples from this stabilisation pond were studied for the presence of fungal species. However, the wastewater at Gulbarga University campus is allowed into a septic tank.

Wastewater samples were collected in a sterile glass container around 6.30 to 7.30 AM on the day of sampling, allowed to settle for 15 min and the supernatant fluid was subjected to physico-chemical and biological analyses. On a single day of sampling, five samples were collected.

Samples from the stabilization pond were collected from 5 different points: one each near the influent and the effluent chambers, and the remaining 3 equidistantly located between these two chambers. Samples were withdrawn from 1 ft below the surface. They were settled for 15 min and the supernatant was subjected to the mycological studies.

The wastewater samples as well as stabilisation pond samples were collected once in a fortnight. The wastewater samples were analyzed for physico-chemical characteristics as per APHA, AWWA and WPCF (1971) as well as for the organic fractions—total carbohydrates (Seifter *et al* 1950), total proteins (Lowry *et al* 1951) and total lipids (Folch *et al* 1957). Serial dilution technique was adopted for the mycological screening of wastewater as well as pond samples. A 0.2 ml sample from 10⁻³ dilution was inoculated by spread plate method onto potato dextrose agar (pH 6.0) containing 300 mg/L streptomycin. Five replicate plates were maintained for each sample and the inoculated plates were incubated at room temperature. Colonies on each plate were identified after sporulation, usually during the 3rd to 6th day of inoculation.

Percentage occurrence of a species was calculated on the basis of total number of colonies of a particular fungus against the total number of colonies of all fungi in a given sampling.

3. Results

The values of physico-chemical characteristics and organic fractions of wastewater and stabilisation pond effluents at Dharwad and wastewater samples at Gulbarga are presented in table 1.

3.1 Fungi in wastewater at Dharwad

Thirteen species belonging to 8 genera were isolated (table 2). *Aspergillus flavus*, *A. niger*, *Penicillium oxalicum*, *Trichosporon* sp., *A. terreus* and *Curvularia lunata* formed the major bulk of the fungal community, and occurred in the same order of percentage of occurrence. However, from the point of consistency of occurrence, *A. niger* occurred with the highest consistency, followed by *A. flavus*, *P. oxalicum*, *Trichosporon* sp., *C. lunata* and *A. terreus*. Amongst other fungal species *Cunninghamella elegans* and *A. niveus* were isolated from many samples but with low percentage of occurrence as well as consistency. Remaining were randomly isolated

	Wastewater at Dharwad	Wastewater at Gulbarga	Stabilization pond effluents*
A. Physico-chemical characteristics:			
pH	6.9-7.6	7.9-9.0	7.9-10.2
Dissolved oxygen	0.0-0.2	0.0-0.2	4.0-15.0
Biochemical oxygen demand (B.O.D.)	250.0-370.0 (314.20)	245.0-500.0 (367.30)	35.0-100.0
Phosphates, dissolved.	3.5-4.6 (4.1)	4.7-6.2 (5.45)	0.8-3.6
Ammonia-N	35.0-49.0 (41.5)	38.0-60.0 (51.5)	5.0-10.0
B. Organic fractions:			
Total carbohydrates	12.936-25.880 (19.509)	18.600-29.440 (24.410)	NA
Total proteins	23.722-51.691 (31.256)	26.700-37.000 (31.590)	NA
Total lipids	32.00-56.00 (46.87)	36.00-47.00 (42.00)	NA

All values in mg/L, except for pH which are in units; numbers in parentheses indicate average values.

*after Patil (1979).

NA = Not analysed.

3.2 Fungi in stabilisation pond at Dharwad

Sixteen fungi belonging to 11 genera were isolated during the course of this study (table 2). The most commonly isolated forms were *A. flavus*, *A. niger*, *P. oxalicum* and *Trichosporon* sp in the order of per cent occurrence. Of the remaining, *A. terreus*, *C. lunata*, *C. elegans*, *Trichoderma viride* and *A. fumigatus* were isolated on most of the occasions though with low per cent occurrence and consistency.

Species of *Aspergillus* dominated over other fungi with $55.52 \pm 3.29\%$ occurrence followed by *P. oxalicum* ($20.28 \pm 2.41\%$ occurrence) and *Trichosporon* sp ($15.02 \pm 2.74\%$ occurrence).

3.3 Fungi in wastewater at Gulbarga

Nineteen species belonging to 12 genera were isolated (table 3). *A. flavus*, *A. niger*, *P. oxalicum* and *Trichosporon* sp were the most commonly isolated forms. However, *A. niger* was the most abundantly occurring species throughout this study. From the point of occurrence as well as consistency, *A. flavus*, *P. oxalicum* and *Trichosporon* sp followed next to *A. niger*.

Aspergillus species dominated over other with $59.31 \pm 6.85\%$ occurrence. Species of *Penicillium* and *Trichosporon* followed next with per cent occurrence of 18.22 ± 4.19 and 14.86 ± 4.79 respectively. Diversity of species of *Penicillium* was more in Gulbarga than in Dharwad.

Organism	Occurrence ± S.D. (%)	Organism	Occurrence ± S.D. (%)
1. <i>Aspergillus flavus</i>	28.01 ± 5.78 (20.63)	1. <i>Aspergillus flavus</i>	27.71 ± 3.51 (12.66)
2. <i>A. niger</i>	24.03 ± 4.56 (18.97)	2. <i>A. fumigatus</i>	1.02 ± 0.66 (64.70)
3. <i>A. niveus</i>	1.05 ± 0.83 (79.00)	3. <i>A. nidulans</i>	0.93 ± 0.86 (92.47)
4. <i>A. terreus</i>	5.09 ± 2.25 (44.20)	4. <i>A. niger</i>	21.59 ± 1.60 (7.41)
5. <i>Cunninghamella elegans</i>	1.95 ± 1.20 (61.53)	5. <i>A. niveus</i>	1.11 ± 1.10 (99.09)
6. <i>Curvularia lunata</i>	3.66 ± 1.46 (39.89)	6. <i>A. terreus</i>	3.16 ± 1.51 (47.78)
7. <i>Fusarium oxysporum</i>	0.51 ± 0.68 (133.33)	7. <i>Candida</i> sp.	0.67 ± 0.49 (73.13)
8. <i>F. solani</i>	0.32 ± 0.69 (215.62)	8. <i>Cladosporium</i> sp.	0.41 ± 0.47 (114.63)
9. <i>Mucor</i> sp.	0.80 ± 0.78 (97.5)	9. <i>Cunninghamella elegans</i>	1.86 ± 0.91 (48.92)
10. <i>Penicillium citrinum</i>	0.75 ± 0.70 (93.33)	10. <i>Curvularia lunata</i>	2.98 ± 1.56 (52.35)
11. <i>P. oxalicum</i>	18.88 ± 4.54 (24.05)	11. <i>Fusarium solani</i>	0.48 ± 0.41 (85.41)
12. <i>Rhizopus nigricans</i>	0.93 ± 0.91 (97.05)	12. <i>Geotrichum candidum</i>	0.93 ± 0.60 (64.51)
13. <i>Trichosporon</i> sp.	14.03 ± 5.59 (39.84)	13. <i>Monilia</i> sp.	0.33 ± 0.42 (127.27)
		14. <i>Penicillium oxalicum</i>	20.28 ± 2.41 (11.88)
		15. <i>Trichoderma viride</i>	1.61 ± 0.81 (50.31)
		16. <i>Trichosporon</i> sp.	15.02 ± 2.74 (18.24)

4. Discussion

In the present study 28 species belonging to 16 genera have been isolated from domestic wastewater and stabilisation pond water samples. Of these, species belonging to 9 genera, viz., *Aspergillus*, *Candida*, *Curvularia*, *Fusarium*, *Geotrichum*, *Mucor*, *Penicillium*, *Rhizopus* and *Trichosporon* were isolated from samples collected at Dharwad as well as Gulbarga. Species of 13 of the genera reported in the present study were earlier recorded from wastewaters and polluted waters in USA by Cooke (1970, 1977) and of 3 genera by Vittal Rao *et al* (1965), at Nagpur, 11 genera by Rai (1981) at Nagpur and 6 from samples at Madras (Vittal and Aravazhi 1981). This indicates the ubiquitous distribution of various fungi in different geographical locations.

Organism	Occurrence \pm S.D. (%)
1. <i>Acrophialophora fusispora</i>	0.29 \pm 0.68 (234.28)
2. <i>Aspergillus flavus</i>	19.15 \pm 4.07 (21.25)
3. <i>A. nidulans</i>	0.63 \pm 0.74 (117.46)
4. <i>A. niger</i>	35.97 \pm 5.97 (16.59)
5. <i>A. niveus</i>	1.22 \pm 0.94 (77.05)
6. <i>A. terreus</i>	2.10 \pm 1.80 (85.71)
7. <i>Candida albicans</i>	0.68 \pm 0.57 (83.82)
8. <i>Curvularia lunata</i>	2.66 \pm 1.49 (56.01)
9. <i>Fusarium equiseti</i>	0.50 \pm 0.57 (114.00)
10. <i>Geotrichum candidum</i>	0.86 \pm 0.56 (65.11)
11. <i>Mucor</i> sp.	0.64 \pm 0.80 (125.00)
12. <i>Penicillium atrinum</i>	0.67 \pm 0.75 (111.94)
13. <i>P. oxalicum</i>	17.11 \pm 4.50 (26.30)
14. <i>P. pinophylum</i>	0.22 \pm 0.43 (195.45)
15. <i>P. simplicissimum</i>	0.22 \pm 0.32 (145.45)
16. <i>Phoma sorghina</i>	0.67 \pm 0.72 (107.46)
17. <i>Rhizopus oryzae</i>	0.89 \pm 0.81 (91.01)
18. <i>Saccharomyces cerevisiae</i>	0.54 \pm 0.68 (125.92)
19. <i>Trichosporon</i> sp.	14.86 \pm 4.79 (32.23)

Numbers in parentheses represent coefficient of variation.

Fungal composition of samples varied in different samplings. Number of species isolated per sampling ranged from 8–12 and 12–14 in wastewater and stabilization pond samples at Dharwad respectively and 7–15 in wastewater samples at Gulbarga. *A. flavus*, *A. niger*, *P. oxalicum* and *Trichosporon* sp were constantly recorded from all the samples examined. *A. flavus* was the predominant form at Dharwad whereas *A. niger* was the predominant fungus in wastewater samples at Gulbarga, which probably reflects upon the latter's tolerance to high temperature levels usually recorded in Gulbarga region. Several species have been recorded with very low percentage of

fusispora, a common soil inhabitant, appears to be related to the mixing up of wastewaters with the nearby soil. This fungus has not been previously reported either from wastewaters or polluted waters elsewhere.

As domestic wastes with high organic load (table 1) undergo biological purification in the stabilization pond, values of Biochemical oxygen demand and nutrients are reduced considerably along with wide diurnal fluctuations in the physico-chemical characteristics (including increases in pH and dissolved oxygen during the afternoon) due to the activities of the pond community *in toto* (Patil 1979). As such, the ambient conditions offered to the inhabitants of wastewaters and to those of stabilisation pond ecosystem differ considerably. The common occurrence of several fungal species in wastewaters as well as in pond reveals their lymaphilic nature. Remaining could be considered as lymaxenes.

The occurrence of fungi in a stabilization pond indicates that they are capable of utilising nutrients from the wastewaters. Cooke and Matsuura (1969) too reported that there was an increase of 5–200 fold in the fungal and yeast populations within an year of operation of a stabilization pond, speaking thereby of the milieu of the pond as a favourable habitat for them. In a stabilization pond ecosystem fungi occupy the same functional status as bacteria, both are effective degraders. Yet in contrast to bacteria which reduce B. O. D. alone but not phosphates and ammonia-N from the domestic wastes (Patil 1979; Hiremath 1984), fungi possess the capability to reduce not only B. O. D., but phosphates and ammonia-N as well (Thanh and Simard 1973; Patil 1979; Hiremath 1984) thus leading to better quality effluents than do bacteria. As such, the presence of fungi and yeasts in waters receiving organic enrichment from one or the other sources, for that matter in any waste treatment system, can not be simply ignored as that of casual contaminants (Cooke 1973).

Studies carried out in this laboratory on the various aspects of fungal purification of domestic wastes as well as interactions of fungi with other representative coinhabitants of the stabilization pond ecosystem will be reported elsewhere.

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References

- APHA, AWWA and WPCF 1971 *Standard Methods for the Examination of Water and Wastewater*, 13th edition, New York
- Cooke W B 1954a Fungi in polluted water and sewage. I. Literature review; *Sewage Ind. Wastes* 26 538–549
- Cooke W B 1954b Fungi in polluted water and sewage. II. Isolation technique; *Sewage Ind. Wastes* 26 661–674
- Cooke W B 1954c Fungi in polluted water and sewage. III. Fungi in a small polluted stream; *Sewage Ind. Wastes* 26 790–794
- Cooke W B 1957 Checklist of fungi isolated from sewage and polluted water; *Sydowia Ann. Mycol.* 1 146–175

- Cooke W B 1970 Fungi in the Lebanon Creek sewage treatment plants and in Turtle Creek, Warren Co., Ohio; *Mycopathol. Mycol. Appl.* **42** 89-111
- Cooke W B 1973 The usefulness of degradative fungi; *Mycopathol. Mycol. Appl.* **51** 199-205
- Cooke W B 1977 Fungi in streams, lakes adjacent soils and sewage treatment systems in the Flathead River basin, Montana; *Northwest Sci.* **51** 172-182
- Cooke W B and Matsuura G 1962 A study of yeast populations in a waste stabilization pond system; *Protoplasma* **57** 163-187
- Cooke W B and Matsuura G 1969 The distribution of fungi in a waste stabilization pond system; *Ecology* **50** 689-694
- Folch J, Lees M and Stanley J H S 1957 A simplified method for the isolation and purification of total lipids from animal tissues; *J. Biol. Chem.* **226** 497-509
- Geldreich E E 1978 Bacterial populations and indicator concepts in feces, sewage, stormwater and solid wastes; in *Indicators of Viruses in Water and Food* (ed) G Berg 51-97
- Hiremath A B 1984 *Studies on the role of fungi in the sewage stabilization pond ecosystem* Ph.D. Thesis, Gulbarga University, Gulbarga
- Lowry O H, Rosebrough N J, Farr A L and Randall R J 1951 Protein measurement with folin phenol reagent; *J. Biol. Chem.* **193** 265-275
- Patil H S 1979 *Studies on the comparative ecology of the various species of algae with a view to select the best for increasing the efficiency of sewage oxidation ponds*, Ph.D. Thesis, Karnatak Univeristy, Dharwad
- Rai R T 1981 Fungal flora of raw sewage; *Indian J. Microbiol.* **21** 84-85
- Rodgi S S and Kanabur V V 1971 The relation of temperature and pH on biochemical oxygen demand in a sewage oxidation pond; *Indian Zool.* **2** 93-104
- Seifter S, Dayton S, Novic B and Muntwyler E 1950 Estimation of glycogen with the anthrone reagent; *Arch. Biochem.* **25** 191-200
- Thanh N C and Simard R E 1973 Biological treatment of domestic sewage by fungi; *Mycopath. Mycol. Appl.* **51** 223-232
- Vittal B P R and Aravazhi D 1981 Preliminary studies on sewage fungi; *Indian J. Environ. Health.* **23** 147-148
- Vittal Rao M, Lakshminarayana J S S, Kumari Ramamani and Bopardikar M V 1965 Role of fungi in sewage purification; *Proc. Symp. Water Pollut. Control 1 Sewage Treatment*, CPHERI, Nagpur 111-118

Genus *Ramaria* in the eastern Himalaya: Subgenus *Laeticolora*—I

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Abstract. In this paper an account of 11 taxa of the genus *Ramaria* (Fr.) Bonorden collected from different localities in the eastern Himalaya and adjoining hills is given. Of the taxa included, *Ramaria conjunctipes*, *Ramaria aralospora* var. *rubella*, *Ramaria xanthosperma*, *Ramaria rubribrunnescens*, *Ramaria flavigelatinosa*, *Ramaria flavigelatinosa* var. *carnismonea* and *Ramaria gelatiniaurantia* are new records for the Himalayas; while *Ramaria rubrogelatinosa*, *Ramaria brevispora*, *Ramaria brevispora* var. *albida* and *Ramaria perbrunnea* were not known earlier from the eastern Himalaya.

Keywords. *Ramaria*; taxonomy; eastern Himalaya.

1. Introduction

Ramaria is the largest genus amongst clavarioid fungi but has scarcely been documented in most parts of the world (Corner 1950; 1970). In India, Thind (1961) and Khurana (1977) reported numerous species of this genus from various localities in the north-western Himalaya. The genus was, however, rather poorly recorded in the eastern Himalaya. During their extensive exploration in the eastern Himalaya, the authors have found the genus abundantly represented in the region.

According to the modern trend, all the taxa belonging to the genus *Ramaria* are treated under 4 subgenera viz. *Ramaria*, *Laeticolora*, *Echinoramaria* and *Lentoramaria*. Of these, subgenus *Laeticolora* Marr and Stuntz is the largest and is characterized by generally massive, fleshy basidiocarps, terrestrial habitat, hyphae with or without clamps and warted basidiospores. All the taxa, which are recorded for the first time from the Himalaya have been described in detail. Additionally, some species from the north-western Himalaya, which were described many years ago by Corner (1966) and Corner *et al* (1958) have not been recorded again from the Himalaya. We have found some of these species in the eastern Himalaya, which here amplifies previous descriptions and documents an extended range of occurrence. The material of all the taxa has been deposited at the Herbarium, Department of Botany, Panjab University (PAN) and at some noted foreign herbaria as indicated. The abbreviations used for herbaria follow Holmgren and Keuken (1974) and the colour standards are according to Kornerup and Wanscher (1967).

2. Subgenus: *Laeticolora* Marr and Stuntz, *Bibliotheca mycol.* 38: 50. 1973

2.1 *Ramaria conjunctipes* (Coker) Corner, *Ann. Bot. Mem.* 1: 567. 1950 (figures 1–6)

Fruit-bodies up to 15 × 5.5 cm, slender, fleshy-waxy, usually occurring in caespitose groups of 2–3, gregarious, light orange to salmon yellow coloured, unchanging on

white; branching 1-2 times; gloeophorous; internodes of the basal branches up to 0.3 cm wide, smooth; axils narrowly U-shaped; tips acute to subacute, mostly bifid, light yellow; flesh concolorous; taste and smell not distinctive.

Hyphal system monomitic; hyphae up to 12 μm wide, without clamps, thin-walled, acyanophilous; ampullaeform swellings prominently ornamented; gloeoplerous hyphae uncommon, up to 3 μm wide, simple or sparsely branched, inflated near the septa, thin-walled, cyanophilous. Basidia up to $67 \times 8.5 \mu\text{m}$, clavate, guttulate, unclamped, 4-spored; sterigmata up to 7 μm long; thick-walled, persistent basidia up to $35 \times 12 \mu\text{m}$, wall up to 1.5 μm thick, granular, 4-spored. Basidiospores $7-8.5(-9.5) \times 4-5(-5.5) \mu\text{m}$, broad-ellipsoid, or oval, uni- to biguttulate; wall slightly thickened, minutely warted, cyanophilous; apiculus up to 0.8 μm long.

Specimen examined: R M Sharda 22239 (PAN; SUCO), on soil under mixed forest, Nawephu, Thimphu, Bhutan, September 18, 1980.

A single collection of this species from Bhutan indicates its rare occurrence in the eastern Himalaya. The species is marked by light orange to salmon coloured fruit-bodies with distinct, acute, light yellow tips; fasciculate habit; absence of clamps and short, broadly ellipsoid, or oval, finely warted basidiospores. It agrees well with the description as given by Corner (1950). The thick-walled, persistent basidia observed in the present collection have not been reported earlier for this species.

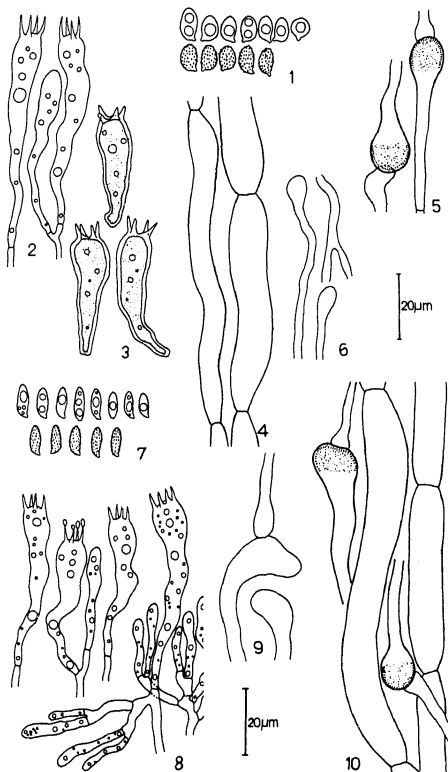
2.2 *Ramaria araiospora* Marr and Stuntz, var. *rubella* Marr and Stuntz, *Bibliotheca mycol.* 38: 57, 1973 (figures 7-10)

Fruit-bodies up to $10 \times 4.5 \text{ cm}$, fleshy, solitary, gregarious, crimson red to deep scarlet red in colour, not fading at maturity, unchanging on bruising; distinct trunk absent, small, white, stubby base (up to 2 cm long) present; branches polychotomous below, dichotomous above, profuse, unequal, in alternating planes, internodes of the basal branches up to 5 mm wide, becoming thinner, shorter and compact upward, smooth; axils narrow; tips subacute, minute, in pairs or clustered, concolorous with the branches; flesh lighter concolorous; taste and smell not distinctive.

Hyphal system monomitic; hyphae up to 11.5 μm wide, without clamps, thin to slightly thick-walled, acyanophilous; ampullaeform swellings ornamented; gloeoplerous hyphae uncommon, up to 4.5 μm wide, simple, inflated near the septa, thin-walled, cyanophilous. Basidia up to $58 \times 8.5 \mu\text{m}$, clavate, guttulate, unclamped, 4-spored; sterigmata up to 5.5 μm long. Basidiospores $7-9.5 \times 2.8-3.5 (-4) \mu\text{m}$, subcylindric, uni- to 3-guttulate; thin-walled, subverruculose, cyanophilous; apiculus up to 0.8 μm long.

Specimens examined: R M Sharda 22444 (PAN), on soil under mixed forest, Shergaon, West Kameng, Arunachal Pradesh, September 6, 1981; R M Sharda 22476 (PAN), on soil under angiospermous forest, 15 km (Rupa-Shergaon road), West Kameng, Arunachal Pradesh, September 10, 1981; R M Sharda 22499 (PAN), on soil under deciduous woods, 3 km (Jamiri-Buragaon road), West Kameng, Arunachal Pradesh, September 13, 1981.

This is a very beautiful fungus and can be easily spotted in the field because of its bright red colour. All the eastern Himalayan collections from Arunachal Pradesh conform well to the description of this variety as given by Marr and Stuntz (1973). The



Figures 1–10. *R. conjunctipes*. 1. Basidiospores. 2. Normal basidia. 3. Thick-walled basidia. 4. Context-hyphae. 5. Ampullaeform swellings. 6. Gloeoplerous hyphae. *R. araiospora* var. *rubella*. 7. Basidiospores. 8. Basidia. 9. Gloeoplerous hyphae. 10. Context-hyphae and ampullaeform swellings.

basidiospores in the eastern Himalayan collections are, however, smaller in size than recorded by Marr and Stuntz (1973) (average up to $9.8 \times 3.6 \mu\text{m}$; range $8\text{--}14 \times 3\text{--}5 \mu\text{m}$).

white to yellowish white or cream coloured; trunk indistinct to distinct, when distinct up to 3.5×1.5 cm, bulbous, yellowish white, with numerous tan red to dull red spots, rubescent on handling; branches profuse, polychotomous throughout, lax, internodes of the lower branches up to 0.6 cm wide, erect, becoming narrower (up to 0.3 cm) upward, smooth; axils broad; tips minute, subacute to obtuse, pale yellow; flesh white; taste and smell not distinctive.

Hyphal system monomitic; hyphae up to $15 \mu\text{m}$ wide, without clamps, sparsely branched, thick-walled (wall up to $1 \mu\text{m}$ thick), acyanophilous; ampullaeform swellings ornamented; gloeoplerous hyphae up to $3 \mu\text{m}$ wide, rarely inflated near the septa, thin-walled, cyanophilous. Basidia up to $86 \times 12 \mu\text{m}$, long-clavate, guttulate, unclamped, 4-spored; sterigmata up to $8.5 \mu\text{m}$ long. Basidiospores average $17.5 \times 4 \mu\text{m}$; range $14.5\text{--}19.5 \times 3.5\text{--}4.5 \mu\text{m}$, subcylindric to cylindric-ellipsoid, uni- to multiguttulate, subsigmoid; wall thin to slightly thickened, warts minute, arranged in rows, cyanophilous; apiculus up to $1.2 \mu\text{m}$ long.

Specimen examined: R M Sharda 22351 (PAN; TENN), on soil under mixed forest, D'Dzong, Paro, Bhutan, August 9, 1981.

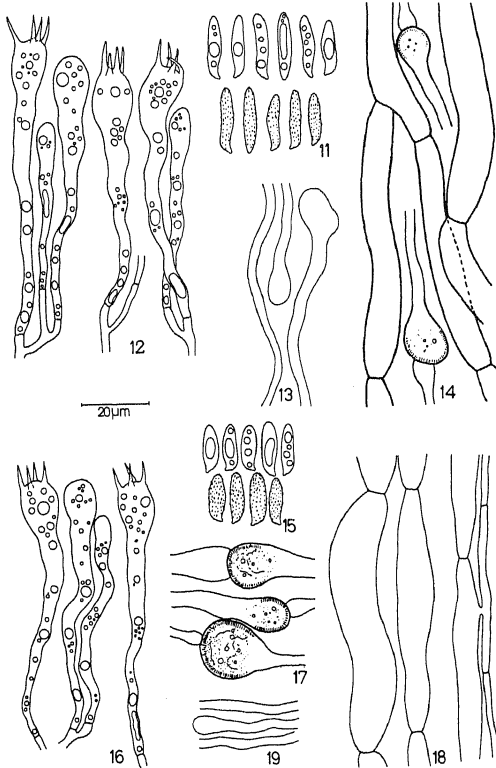
Diagnostic features of this species are the yellowish white to cream colour of the fruit-bodies with conspicuous dull red to tan red spots on the basal part; thick-walled, clampless hyphae; and subsigmoid, minutely warted, long basidiospores. But for the slightly larger basidiospores the Bhutan collection closely resembles the description given by Corner (1950, 1970).

2.4 *Ramaria rubribrunnescens* Marr and Stuntz, *Bibliotheca mycol.* 38: 111. 1973 (figures 15–19)

Fruit-bodies up to 21.5×9 cm, fleshy, solitary, scattered closely, pale yellow to cream coloured; trunk up to 3×0.6 cm, in other fruit-bodies trunk consists of 3–4 subfasciculate branches, white, trunk or lower part of branches with numerous scattered red spots or sometimes the basal branches staining reddish brown, perhaps vinescent on handling; branching profuse, polychotomous below, dichotomous above, internodes of the lower branches up to 0.5 cm wide, smooth, becoming thinner, shorter and compact upward; axils U-shaped or narrow; tips minute, obtuse, swollen, light yellow; flesh white to paler concolorous; taste and smell not distinctive.

Hyphal system monomitic; hyphae up to $14.5 \mu\text{m}$ wide, without clamps, sparsely branched, wall slightly thickened, acyanophilous; ampullaeform swellings conspicuously ornamented; gloeoplerous hyphae rare, up to $3 \mu\text{m}$ wide, simple, thin-walled, cyanophilous. Basidia up to $82 \times 12.5 \mu\text{m}$, clavate, guttulate, unclamped, 4-spored; sterigmata up to $7 \mu\text{m}$ long. Basidiospores $12.5\text{--}14(-15.5) \times 4\text{--}5(-5.5) \mu\text{m}$, subcylindric to ellipsoid, uni- to multiguttulate; thin-walled, subverruculose to verrucose, cyanophilous; apiculus up to $1 \mu\text{m}$ long.

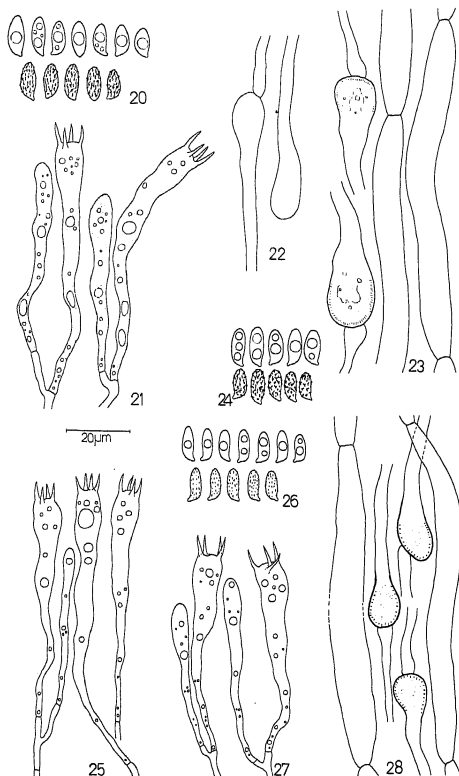
Specimens examined: R M Sharda 22240 (PAN), on soil under mixed forest, Nawepchu, Thimphu, Bhutan, September 18, 1980; R M Sharda 22266 (PAN), on soil under mixed forest of *Picea*, *Abies* and *Rhododendron*, Chankaphug, Thimphu, Bhutan, September 23, 1980.



Figures 11–19. *R. xanthosperma*. 11. Basidiospores. 12. Basidia. 13. Gloeoplerous hyphae. 14. Thick-walled context-hyphae and ampullaeform swellings. *R. rubribrunnescens*. 15. Basidiospores. 16. Basidia. 17. Ampullaeform swellings. 18. Context-hyphae. 19. Gloeoplerous hyphae.

Characteristics that separate *R. rubribrunnescens* from the similar species are massive basidiocarps, pale yellow to cream in colour; numerous dull red or tan red spots (perhaps vinescent) on the trunk and basal branches; hyphae without clamps; and subcylindric to ellipsoid, minutely warted basidiospores. Both the eastern Himalayan collections resemble closely the description given by Marr and Stuntz

Fruit-bodies up to 10×6 cm, medium sized, solitary, gregarious, yellowish white to light yellow, unchanging on bruising; distinct trunk absent, instead the base consisting of several branches, white, occasionally with dull violet spots observed on these



Figures 20–28. *R. flavigelatinosa*. 20. Basidiospores. 21. Basidia. 22. Gloeoplerous hyphae. 23. Context-hyphae and ampullaeform swellings. *R. flavigelatinosa* var. *carnismonea*. 24. Basidiospores. 25. Basidia. *R. gelatiniaurantia*. 26. Basidiospores. 27. Basidia. 28. Context-hyphae and ampullaeform swellings.

branches, becoming polychotomous, lax, internodes of the basal branches up to 0.5 cm wide, smooth, becoming thinner and shorter in the subsequent branches; axils wide open; tips single or dichotomous, subacute to obtuse, sunflower yellow; flesh paler concolorous, watery; texture gelatinous when fresh, becoming tough after drying; taste and smell not distinctive.

Hyphal system monomitic; hyphae up to $13.5\ \mu\text{m}$ wide, without clamps, wall thin to slightly thickened, acyanophilous; ampullaeform swellings ornamented; gloeoplerous hyphae common, up to $4\ \mu\text{m}$ wide, inflated near the septa, thin-walled, cyanophilous. Basidia up to $70 \times 10\ \mu\text{m}$, clavate, granular-guttulate, unclamped, 4-spored; sterigmata up to $7.5\ \mu\text{m}$ long. Basidiospores average $10.5 \times 4.5\ \mu\text{m}$; range $8.5\text{--}11(-12) \times 4\text{--}5(-5.5)\ \mu\text{m}$, broad-ellipsoid, uni- to multiguttulate; wall slightly thickened, verruculose, warts in oblique rows, cyanophilous; apiculus up to $1\ \mu\text{m}$ long.

Specimens examined: R M Sharda 22103 (PAN), on soil under *Pinus kesiya* forests, Elephant falls, Shillong, Meghalaya, September 18, 1979; R M Sharda 22132 (PAN), on soil under *P. kesiya* forest, Shillong Peak, Shillong, Meghalaya, September 21, 1979; R M Sharda 22142 (PAN), on soil under *P. kesiya* forest, Sweet falls, Shillong, Meghalaya, September 23, 1979; R M Sharda 22217 (PAN), on soil under angiospermous forest, Batasi, Darjeeling, West Bengal, August 28, 1980; R M Sharda 22310 (PAN), on soil under broad-leaved forest, Bunakha, Chimakothi, Bhutan, July 29, 1981.

The species is fairly common in the eastern Himalaya. All these collections closely resemble the description of this species as given by Marr and Stuntz (1973) but for the larger fruit-body size in the collections made from Washington (up to $5\text{--}14 \times 3\text{--}24\ \text{cm}$; fide Marr and Stuntz 1973).

2.6 *Ramaria flavigelatinosa* Marr and Stuntz, var. *carnisalmonea* Marr and Stuntz, *Bibliotheca mycol.* 38: 83. 1973 (figures 24–25)

Variety *carnisalmonea* resembles *R. flavigelatinosa* var. *flavigelatinosa* in all other macroscopic and microscopic features but for the sole characteristic of salmon colour of the context.

The only eastern Himalayan collection made from Bhutan agrees closely with the concept of this variety as given by Marr and Stuntz (1973). It is marked by light yellow colour of the fruit-bodies with orange or pinkish tinge in the upper parts; yellow tips; salmon colour of the context; gelatinous consistency of the fresh specimens; lack of clamps and subcylindric to ellipsoid, distinctly warted basidiospores, $9.5\text{--}11.5 \times 4\text{--}5\ \mu\text{m}$.

Specimen examined: R M Sharda 22238 (PAN; SUCO), on soil under mixed forest, Nawephu, Thimphu, Bhutan, September 19, 1980.

2.7 *Ramaria gelatiniaurantia* Marr and Stuntz, *Bibliotheca mycol.* 38: 93. 1973 (figures 26–28)

Fruit-bodies up to $9 \times 8\ \text{cm}$, fleshy, gregarious or scattered, orange white to deep orange, colour unchanging on bruising; distinct trunk absent, instead base consisting of 4–5 connate, primary branches, white to orange white; branching profuse,

thinner, shorter and compact in the upper branches; axils narrow; tips minute, mostly acute to rarely subacute, deep orange; flesh lighter concolorous, watery; texture gelatinous, especially the basal part more gelatinized than the rest of the fruit-body, drying tough and brittle; taste and smell not distinctive.

Hyphal system monomitic; hyphae up to $12.5(-17) \mu\text{m}$ wide, without clamps, sparsely branched, wall slightly thickened, acyanophilous; ampullaeform swellings prominently ornamented; gloeoplerous hyphae not observed. Basidia up to $65 \times 11 \mu\text{m}$, clavate, guttulate, weakly cyanophilous, unclamped, 4-spored; sterigmata up to $7 \mu\text{m}$ long. Basidiospores average $9.5 \times 4 \mu\text{m}$; range $8.5-10(-11) \times 3.8-4.5 \mu\text{m}$, subcylindric, uni- to biguttulate; wall thin to slightly thickened, verruculose, cyanophilous; apiculus up to $1 \mu\text{m}$ long.

Specimen examined: R M Sharda 22121 (PAN; SUCO), on soil under *P. kesiya* forest, Risa colony, Shillong, Meghalaya, September 20, 1979.

This collection from Shillong conforms well to the description of this species as given by Marr and Stuntz (1973). However, the fruit-bodies measure up to $6-22 \times 4-11 \text{ cm}$ and possess gloeoplerous hyphae in the context in the Washington collection, whereas our collection measures up to $9 \times 8 \text{ cm}$ and lacks gloeoplerous hyphae in the context.

Professor R H Petersen sent us two collections, No. 45845 (TENN; on needle duff under hemlock forests, Mt. Hood Nat'l forest, Green Cyn CAmpgrd Road, Oregon, USA, October 20, 1984) and No. 45851 (TENN; on needle duff under mixed conifer forest, vicinity of Humptulip, Jefferson Co., Washington, USA, October 6, 1984) which were examined by us. The Shillong collection closely resembles these US collections.

2.8 *Ramaria rubrogelatinosa* Corner and Thind, Trans. Br. mycol. Soc. 49: 110. 1966

Fruit-bodies up to $18 \times 9 \text{ cm}$, fleshy, gregarious to scattered, pink to light red in colour, fading to cream or white in mature specimens with age, unchanging on bruising; distinct trunk absent, instead small, stubby base present, buried in soil, white; branching starting immediately at the ground level, profuse, polychotomous below, dichotomous above, internodes of the basal branches thick, up to 1 cm wide, becoming thinner and compact upward; axils acute; tips minute, subacute to obtuse, deep red, colour not fading with age; flesh white to paler concolorous; texture gelatinous, drying hard, brittle; taste and smell not distinctive.

Hyphal system monomitic; hyphae up to $12(-15) \mu\text{m}$ wide, without clamps, wall thin, acyanophilous; ampullaeform swellings conspicuously ornamented; gloeoplerous hyphae uncommon, up to $4 \mu\text{m}$ wide, simple to forked, inflated near the septa, thin-walled, cyanophilous. Basidia up to $70 \times 8.5 \mu\text{m}$, clavate, unclamped, weakly cyanophilous, 4-spored; sterigmata up to $6.5 \mu\text{m}$ long. Basidiospores $7.5-9.5(-10.5) \times 4-4.5(-5) \mu\text{m}$, ovoid to broad-ellipsoid, uni- to multiguttulate; wall slightly thickened, rugulose to subverruculose, cyanophilous; apiculus up to $0.7 \mu\text{m}$ long.

Specimens examined: R M Sharda 22071 (PAN), on soil under angiospermous forest, Takdah Cantt., Darjeeling, West Bengal, August 31, 1979; R M Sharda 22137 (PAN), on soil under angiospermous forests, Shillong Peak, Shillong, Meghalaya, September 21, 1979; R M Sharda 22179 (PAN), on soil under angiospermous forest, Takdah

features of this species are the pink to light red colour of the fruit-bodies, fading to cream or white with age; tips deep red and not fading at maturity; gelatinous consistency; total lack of clamps and rugulose to subverruculose basidiospores.

2.9 *Ramaria brevispora* Corner, Thind and Dev, Trans. Br. mycol. Soc. 41: 203. 1958

Fruit-bodies up to 15 × 6 cm, fleshy, gregarious to scattered, light yellow, colour unchanging on bruising; trunk usually indistinct, when distinct up to 2.5 × 0.6 cm, smooth, yellowish white; branching profuse, polychotomous below, dichotomous above, internodes of the basal branches up to 0.4 cm wide, becoming thinner, shorter and compact in the upper branches, smooth; axils wide open; tips minute, dichotomous or multiple, blunt, deep yellow; flesh white; taste and smell not distinctive.

Hyphal system monomitic; hyphae up to 10.5 (–14) μ m wide, without clamps, sparsely branched, thin-walled, acyanophilous; ampullaeform swellings ornamented; gloeoplerous hyphae not observed. Basidia up to 56 × 8.5 μ m, clavate, guttulate, weakly cyanophilous, unclamped, 4-spored; sterigmata up to 6 μ m long. Basidiospores 5.5–7 × 4–5 μ m, subglobose to sublacrimiform, uniguttulate; wall slightly thickened, finely warted, warts more conspicuous toward the distal end of the basidiospores, cyanophilous; apiculus up to 0.7 μ m long.

Specimens examined: R M Sharda 22115 (PAN), on soil under angiospermous forest, Mawphlang, Shillong, Meghalaya, September 19, 1979; R M Sharda 22457 (PAN), on soil under mixed forest, Shergaon-Kalaktang road, West Kameng, Arunachal Pradesh, September 7, 1981; R M Sharda 22491 (PAN), on soil under broad-leaved forest, 3 km (Jamiri-Buragaon road), West Kameng, Arunachal Pradesh, September 13, 1981.

During our exploration in the eastern Himalaya, we collected this fungus thrice under predominantly angiospermous forests. These collections resemble closely the holotype (at PAN). Distinguishing features of this species are the light yellow coloured fruit-bodies having deep yellow, blunt tips; total lack of clamps; and subglobose to sublacrimiform, finely warted, 5.5–7 × 4–5 μ m basidiospores. This is the second report of the occurrence of this species from the Himalaya after it was reported in 1958.

2.10 *Ramaria brevispora* Corner, Thind and Dev, var. *albida* Corner, Thind and Dev, Trans. Br. mycol. Soc. 41: 204. 1958

This variety was described by Corner *et al* (1958) from Mussoorie (UP) and like *R. brevispora* it was also known only from the type locality previously. It resembles *R. brevispora* in all other details but for the different colour of the fruit-bodies which is marble white in the lower branches and tan white in the upper branches.

A single collection of this variety from Shergaon in Arunachal Pradesh differs from

the holotype (at PAN) in possessing gloeoplerous hyphae in the context. In all other details it closely resembles the description as given by its authors and Khurana (1977).

Specimen examined: R M Sharda 22458 (PAN), on soil under mixed forest, Shergaon, West Kameng, Arunachal Pradesh, September 7, 1981.

2.11 *Ramaria perbrunnea* Corner and Thind, Trans. Br. mycol. Soc. 49: 109. 1966

Fruit-bodies up to 12×5 cm, fleshy, gregarious or scattered, light brown to dark brown, colour unchanging on bruising; distinct trunk absent, instead small, subfasciculate base present, up to 2×0.8 cm, bulbous, white; branching profuse, polychotomous below, dichotomous above, internodes of the basal branches stout, up to 0.5 cm wide, rugulose, internodes shorter and thinner in the subsequent branches; axils narrow to U-shaped; tips subacute to obtuse, concolorous; flesh white; texture brittle friable after drying; taste slightly bitter, smell not distinctive.

Hyphal system monomitic; hyphae up to $10.5 \mu\text{m}$ wide, without clamps, thin to slightly thick-walled, acyanophilous; ampullaeform swellings conspicuously ornamented; gloeoplerous hyphae not observed. Basidia up to $81 \times 9 \mu\text{m}$, clavate, guttulate, unclamped, 4-spored; sterigmata up to $7 \mu\text{m}$ long. Basidiospores average $11.2 \times 5.5 \mu\text{m}$; range $10.5\text{--}12(-13.5) \times 4.5\text{--}6 \mu\text{m}$, broadly ovoid, uni- to multiguttulate; wall slightly thickened, surface prominently warted, warts coarse, irregular, dense, cyanophilous; apiculus up to $1.2 \mu\text{m}$ long.

Specimen examined: R M Sharda 22246 (PAN), on soil under mixed forest, Begana, Thimphu, Bhutan, September 19, 1980.

The Bhutan collection resembles well the holotype (at PAN) examined by us. The species is marked by medium sized fruit-bodies arising from subfasciculate base; stout branches; brown colour; slightly bitter taste and uni- to multiguttulate, coarsely warted basidiospores.

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References

- Corner E J H 1950 A monograph of *Clavaria* and allied genera; *Ann. Bot. Mem.* 1 740
Corner E J H 1966 Species of *Ramaria* (Clavariaceae) without clamps; *Trans. Br. Mycol. Soc.* 49 101–113
Corner E J H 1970 Supplement to "A monograph of *Clavaria* and allied genera"; *Beih. Nova Hedwigia* 33 299
Corner E J H, Thind K S and Dev S 1958 The Clavariaceae of the Mussoorie Hills (India) IX. *Trans. Br. Mycol. Soc.* 41 203–206

- Khurana I P S 1977 *Studies on the clavarioid fungi of India*, Ph.D. thesis. Panjab University, Chandigarh
- Kornerup A and Wanscher J H 1967 *Methuen handbook of colour*, 2nd edition (London: Methuen & Co. Ltd.) p 243
- Marr C D and Stuntz D E 1973 *Ramaria* of Western Washington; *Bibliotheca Mycol.* **38** 232
- Thind K S 1961 *The Clavariaceae of India*, (New Delhi: Indian Council Agri. Res.) p 197

Comparative study on the seed of two major pulses vis-a-vis their common adulterant

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Abstract. Comparative morphological and anatomical investigations on the seed of two major pulses *Cicer arietinum* L. (Desi Chana) and *Cajanus cajan* (L.) Millsp. var. *bicolor* DC. (Arhar)—vis-a-vis their common adulterant (*Lathyrus sativus* L. (Khesari Dal)), have yielded several features of value in pin-pointing adulteration in the market samples. Being basically similar in constitution, the seed coat in all the three taxons-comprises (i) a cuticle, (ii) an epidermis, (iii) a hypodermis, and (iv) a mesophyll. While the cuticle is 'thick and wavy' in *Cicer arietinum* and 'thin and smooth' in *Cajanus cajan*, it is notably 'thick and dentate' in *Lathyrus sativus*. The macrosclereids constituting the epidermis and osteosclereids forming the hypodermis provide a set of contrasting features with regard to their shape, size, thickness and lumen/corrugation thereof.

Keywords. Testa; macrosclereids; osteosclereids; lumen; corrugation.

1. Introduction

A good deal of work is on record regarding the embryology and morphology of seed in Leguminosae (Pammel 1899; Corner 1951, 1976; Esau 1965, 1977; Davis 1966; Chowdhury and Buth 1970; Vaughan 1970; Purseglove 1974; Kochhar 1981). Similar studies on the fruits/seeds of some angiosperms have also been made by Gupta and Lamba (1981, 1983, 1984), Lamba and Gupta (1981a, b) and Gupta (1982). A comparative morphological/anatomical study on the seed of two major pulses—*Cicer arietinum* L. (Brown or Desi Chana), *Cajanus cajan* (L.) Millsp. var. *bicolor* DC. (Arhar)—vis-a-vis that of their major adulterant *Lathyrus sativus* L. (causing lathyrism), not attempted earlier, was found most useful in pin-pointing adulteration rampant in the Indian Markets (Jacob 1976). The results of the study are reported here.

2. Materials and methods

Pure seeds of the two pulses as also of their adulterant (*L. sativus*) were collected from Botanical Garden, Kurukshetra University. The samples collected during market survey were subjected to critical analysis, macroscopically as well as microscopically (using stereo-scopic binoculars and light microscope). Free hand sections/microtome sections were cut (after subjecting the seed to glycerol-water treatment). These were stained with safranin light green combinations. Temporary mounts were made in 5% glycerol medium after staining the sections with 1% aqueous safranin. In order to study the anatomical constitution of the field ripe seeds/seed coat, the same were also

While the seeds in the three taxons resemble in being derived from a bitegmic and crassinucellate ovule (Singh 1964; Behl 1976), they differ in the ovule curvature—the same being campylotropous in *Cajanus cajan* and anacampylotropous in *C. arietinum* and *L. sativus*. Though the seed/decoated cotyledons and fragments apparently look more or less alike (figures 1–4), these were found to possess several features of distinction when put to critical examination. Table 1 depicts some exomorphic features of the seed/cotyledons of the three taxons.

3.2 Anatomical features

Having a basically similar constitution, the ripe seed in all the aforesaid three taxons consists of the testa, enveloping a typically dicotyledonous embryo—the endosperm being represented only by tattered remains that lie closely appressed to the testa in transection (Corner 1976). The testa which is the metamorphosed outer integument in toto (figures 5, 7, 9) comprises: (i) a cuticle, (ii) an epidermis, (iii) a hypodermis, and (iv) a mesophyll. The detailed anatomical structure of the seed coat in the three taxons has been observed:

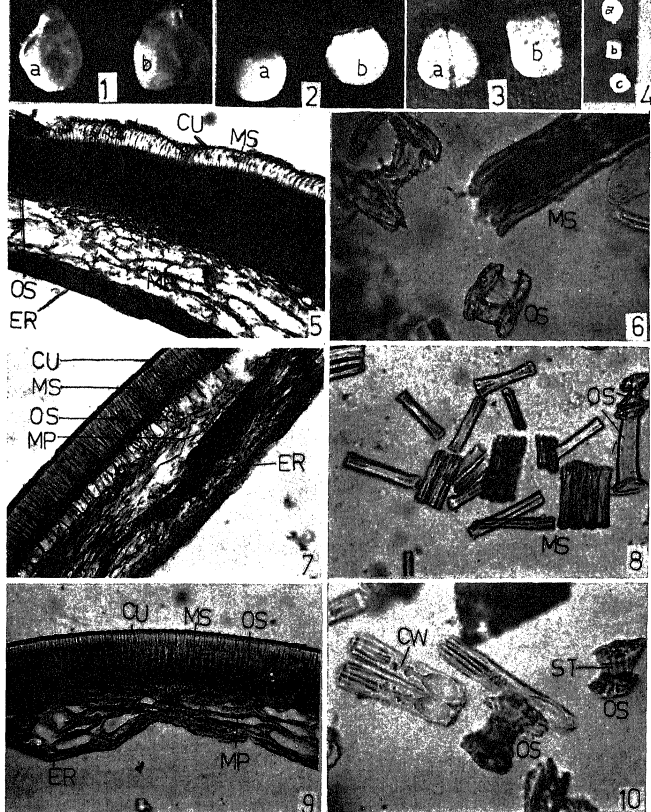
3.2.1 Cuticle: The cuticle is characteristically thick, rough and undulated in *C. arietinum*, relatively thin and smooth in *C. cajan* and appreciably thick with a dentate surface in *L. sativus* (figures 5, 7, 9).

3.2.2 Epidermis: In all the three taxons, the epidermis is a single layer of ‘palisade-like’ cells which though similar in function differ significantly in their structure and orientation (figures 5, 7, 9). Technically, known as ‘macroscleireids’ these cells are lignified and appear dark-brown to black due to heavy deposition of tanniferous contents. However, in *L. sativus* as also in *C. cajan*, the macroscleireids are of uniform size—in contrast to those of *C. arietinum* wherein alternative groups of taller and dwarfer macroscleireids characteristically constitute the epidermis (figures 5, 7, 9).

The comparative structure of individual sclereids in each of the three taxons provides several significant features of distinction. Thus, while the macroscleireids in *C. arietinum* are ‘elongated, pointed and bent at the lower end’ (figures 6, 17, 18), ‘simply column like’ in *C. cajan* (figures 8, 14), those in *L. sativus* are characteristically ‘spool shaped’ (figures 10, 11). Furthermore, their respective lumens are ‘narrow at both ends and sinuous in the middle’ in *C. arietinum* (figures 6, 17, 18), ‘bulbous at the base being narrow above’ in *C. cajan* (figures 8, 14) and ‘broad and bulbous at base narrowing above’ in *L. sativus* (figures 10, 11). There is also clear difference in the position of ‘corrugation of inner faces’ of macroscleireids: ‘near the upper end’ in *C. arietinum* (figures 17, 18), ‘more or less absent’ in *C. cajan* (figures 8, 14) and ‘near the middle region’ in *L. sativus* (figures 10, 11).

Table 2 gives the relevant comparative data regarding the macroscleireids:

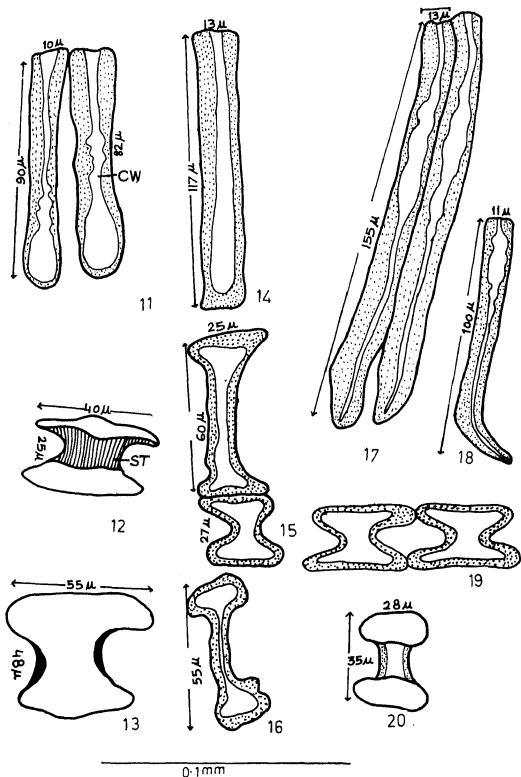
3.2.3 Hypodermis: The layer subjacent to epidermis is ‘hypodermis’. In all the three pulses, it is made of osteosclereids (hour glass cells of Esau 1977). It is single-



Figures 1-10. 1-3. Photographs of 'whole seed' of *C. arietinum*, *C. cajan* and *L. sativus* in front and side views respectively $\times 3.5$. 4. Decoated cotyledon of *C. arietinum*; *L. sativus* and *C. cajan* respectively $\times 2$. 5, 7, 9. Photomicrographs in transection of the ripe seed of *C. arietinum*, *C. cajan* and *L. sativus* respectively $\times 500$. 6, 8, 10. Macroscleireids and osteosclereids in the seed coat of *C. arietinum* ($\times 2000$), *C. cajan* ($\times 500$) and *L. sativus* ($\times 2000$), respectively. CH, Chalaza; CU, cuticle; CW, corrugated wall; ER, tattered remains of endosperm; HI, hilum; MP, mesophyll; MS, macrosclereids; OS, osteosclereids; ST, striations.

Table 1. Major exomorphic features of the seed in *C. arietinum*, *C. cajan* and *L. sativus*

Plant	Seed colour	Seed size (mm)	Shape of seed	Colour; shape and size (mm) of cotyledon	Size and position of hilum	Seed surface
<i>C. arietinum</i> L. (Brown or Desi Chana)	Dark-brown to reddish brown to blackish	7-9 × 4-5	Angular and oblong with a prominent beak	Yellowish; thick globose 5-6.5 × 4-5 × 2-3	1.9 × 1.5 mm in sunken pouch, near the beak, below the level of seed surface, white covering present	Wrinkled (undulose) and blistered, spotted
<i>C. cajan</i> (L.) Millsp. var. <i>bicolor</i> DC. (Arhar)	Reddish brown or silvery white	6-7 × 4-5	Orbicular and compressed with one edge flattened	Yellow; D-shaped relatively thin than * <i>C. arietinum</i> 5-6 × 4-5 × 0.95	3.5 × 2.0 mm Elliptical, raised above the surface, covered with white tissue	Smooth, shiny and spotted
<i>L. sativus</i> L. (Khesari Dal)	Yellowish brown to brown	6-7 × 5	Wedge shaped and angular	Yellow; wedge shaped relatively thin than * <i>C. arietinum</i> 4.5 × 3.5-4 × 1.0	2 × 1.0; Elliptical in level with seed surface and white hard covering absent.	Smooth and moist



Figures 11–13. *L. sativus*. 11. Macrosclereids showing corrugated inner wall; 12–13. Osteosclereids with longitudinal striations, and in lateral view respectively; 14–16. *C. cajan*; 14. Macrosclereid. 15. Osteosclereids in two layer. 16. A typical osteosclereid. 17–20. *C. arietinum*. 17–18. Taller and dwarfed macrosclereids. 19. Two osteosclereids in lateral view. 20. A single osteosclereid. CW, corrugated wall; OS_I, osteosclereid of Ist layer; OS_{II}, osteosclereid of II layer; ST, striations.

layered in *C. arietinum* and *L. sativus* (figures 5, 9) and contains 2 layers in *C. cajan* (figures 7, 8). The osteosclereids are 'I' shaped in *C. arietinum* (figures 6, 19, 20), bone-shaped in *C. cajan* (figures 8, 15, 16) and possess longitudinal striations with radial

Taxon	Shape	Size in (μ)	Lumen	Corrugation	Colour
<i>C. arietinum</i>	Elongated, being bent at lower end	Taller ones: 155–170 × 12–18 Dwarfer ones: 100–110 × 11–16	Narrow at both ends but sinuous in the middle	Present (near the upper end)	Dark-brown to black
<i>C. cajan</i>	Column-like	50.0–110 × 10–24	Bulbous at base tapering above	More or less absent	Greyish-brown
<i>L. sativus</i>	Spool shaped	75–90 × 10–12.5	Broad and bulbous (narrow above but constricted in the middle)	Present (extending from basal to middle region)	Reddish-brown

other two pulses (figures 6, 8, 10). The measurements of osteosclereids are 35–45 × 25–40 μ for *C. arietinum*, 55–100 × 25–50 μ and 27 × 27 μ for the two layers in *C. cajan* and 25–48 × 40–55 μ for *L. sativus*.

3.2.4 Mesophyll: Lying inner to hypodermis, the mesophyll consists of aerenchymatous cells. This layer, too, provides an interesting contrast—being 3 to 4 layered in *C. arietinum*, 5 to multilayered in *C. cajan* and 2 to 3-layered in *L. sativus* (figures 5, 7, 9).

4. Discussion

The problem of adulteration of food materials is very much rampant in the various Indian markets (Jacob 1976). Random samples of the two major pulses, *C. arietinum* and *C. cajan*—in the 'whole seed form' and as 'decoated/broken cotyledons'—were found adulterated with 'whole seed'/'decoated cotyledons' of *L. sativus*. The said samples were found adulterated to the tune of 5–8%. The present investigations, comprising macro- as well as microscopic studies, have yielded data of significant potential importance in analysing such adulterated samples.

The seed in the three taxa, *C. arietinum*, *C. cajan* and *L. sativus*, all belonging to family Fabaceae, possesses a basically similar constitution. However, the seed of *C. arietinum* is notably brown to black, with angular or oblong shape and that of *C. cajan* is silvery to reddish brown (showing different shades of red or yellow) having orbicular and compressed shape in contrast to the seed of *L. sativus* which is yellowish brown, mottled, wedged and angled. Yet another remarkable exomorphic feature is the position of hilum which in case of *C. arietinum* is located in a sunken pouch with a 'white hard covering', whereas in *C. cajan* it is raised above the level of seed surface and exhibits the 'significant white covering' but is notable for its being at level with the seed surface and showing absence of the 'white hard tissue' in *L. sativus*. Furthermore, the cotyledons which are yellowish and globose in *C. arietinum* are yellow and D-shaped in

the seed surface which is wrinkled and nonspotted in *C. arietinum*, smooth and non-spotted in *C. cajan* but characteristically smooth and mottled in *L. sativus*.

Comparative structure of individual sclereids has also provided valuable features of distinction.

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References

- Behl H M 1976 *Developmental anatomy of seed and fruits of Phaseoleae and Viceae (Fabaceae)*, Ph.D. Thesis, Rajasthan University, Jaipur
- Chowdhury K A and Buth G M 1970 Seed coat structure and anatomy of Indian pulses; in *New Res. in Pl. Anatomy Suppl 1. Bot. J. Linn. Soc.* 63 169-79
- Corner E J H 1951 The leguminous seed; *Phytomorphology* 1 1-34
- Corner E J H 1976 *The seeds of dicotyledons* (Cambridge: Cambridge University Press) vols. 1 and 2
- Davis G L 1966 *Systematic Embryology of the Angiosperms* (New York: John Wiley and Sons Inc)
- Esau K 1965 *Plant Anatomy* (New Delhi: Wiley Eastern Pvt. Ltd.)
- Esau K 1977 *Anatomy of seed plants* (New York: John Wiley and Sons Inc.)
- Gupta Veena 1982 *Anatomical and Morphological Studies on the fruits and seeds of some economically important Angiosperm*, Ph.D. Thesis, Kurukshetra University, Kurukshetra
- Gupta Veena and Lamba L C 1981 Sclereids in endocarp of *Rauvolfia serpentina* L. Benth ex Kurz. *Proc. Indian Acad. Sci.* B90 79-84
- Gupta Veena and Lamba L C 1983 Comparative investigation on the seed of some Papaveraceae; *Abst. Annual Conf. Soc. Advancement of Botany*, Haryana Agricultural University, Hissar
- Gupta Veena and Lamba L C 1985 Structure and development of seed in *Eschscholzia californica* Cham; *Acta Bot. Indica* 13 (in press)
- Jacob T 1976 *Food Adulteration* (Delhi: The Macmillan and Co. of India)
- Kochhar S L 1981 *Economic Botany in the Tropics*; (New Delhi: Macmillan India Ltd.)
- Lamba L C and Gupta Veena 1981a SEM study of seed surface in *Argemone mexicana* and *Brassica campestris* var. Brown Toria; *Curr. Sci.* 50 738-40
- Lamba L C and Gupta Veena 1981b Surface studies on seed coat of *Argemone mexicana* L.; *Sci. Cult.* 74 109-110
- Pammel L H 1899 Anatomical characters of the seed of Leguminosae; *Trans. Acad. Sci. St. Louis* 9 91-275
- Purseglove J W 1974 *Tropical Crops Dicotyledons* (London: Longman Group Ltd.)
- Singh B 1964 *Development and structure of Angiosperm seed—I Review of Indian work*; *Bull. Nat. Bot. Gdns. Lucknow* 89 1-115
- Vaughan J G 1970 *The structure and utilization of oilseeds* (London: Chapman and Hall Ltd.)

Contribution to the embryology of *Calamintha umbrosa* Benth.

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Abstract. The anther development confirms to the dicotyledonous type. The tapetal cells are binucleate, glandular and dimorphic. The pollen tetrads are tetrahedral and decussate. The pollen grains are shed at 2-celled stage. The development of embryo sac is of the polygonum type. A weak hypostase is present. The micropylar haustorium develops lateral diverticulum. The endosperm is *ab initio* cellular and its development conforms to 'stachys type'. The embryo development follows *Mentha* variation of onagrad type. The seed coat is 1-layered. The pericarp consists of 3 zones. The outer epidermis is non-mucilaginous. At maturity, the hypodermal layer of inner epidermis forms the main protective layer. Seed is endospermic.

Keywords. *Calamintha umbrosa*; sporogenesis; gametogenesis; embryogeny; seed coat; pericarp.

Introduction

Calamintha umbrosa Benth. belongs to sub-tribe, Melisseae and the tribe Satureineae of the family Lamiaceae. Schnarf (1931) and Davis (1966) have reviewed the earlier embryological work on the family. Casual work on embryology of certain members of the tribe Satureineae has been done by Ruttle (1931 and 1932), Jaitly (1968), Jaitly *et al* (1968) and Santha Kumari (1976). The present study deals with the embryology and seed coat and pericarp structure of *C. umbrosa*.

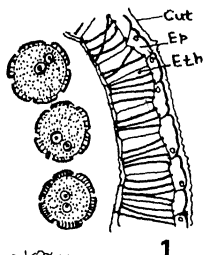
Materials and methods

The materials were collected from National Botanical Garden, Darjeeling (alt. 7000 ft from sea level) during October, 1978 and fixed in FAA. Customary methods of dehydration, infiltration and embedding were followed. Sections cut at a thickness of 10 μ m were stained with iron alum-haematoxylin using safranin as counter stain.

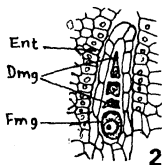
Observations

1 Microsporogenesis and male gametophyte

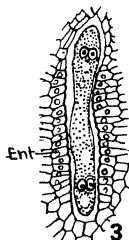
The anther is tetrasporangiate and develops according to the dicotyledonous type.



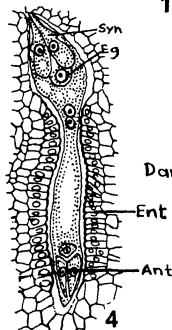
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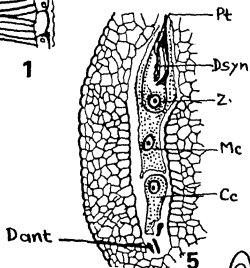
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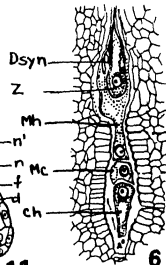
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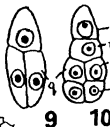
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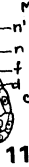
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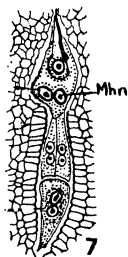
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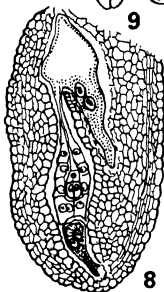
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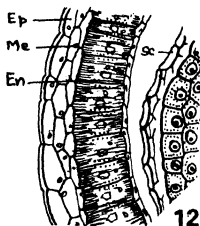
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7



8



12

The ovule is anatropous, unitegmic and tenuinucellate. The archesporium is single celled. The megaspore mother cell divides meiotically resulting in the formation of a linear tetrad of megaspores. The chalazal one of which develops into an 8-nucleate embryo sac of the polygonum type (figures 2-4). The endothelium develops at the stage of megaspore mother cell persists upto the late endosperm stage (figures 2-8). An ill developed hypostase is present.

3.3 Endosperm

The first division of the primary endosperm nucleus is accompanied by a transverse wall forming a small chalazal chamber and a large micropylar chamber (figure 5). The nucleus in the chalazal chamber divides once more without any wall formation organising two nucleate chalazal haustorium (figure 7). In a mature fruit the latter extends and establishes contact with conducting strands (figure 8). The division in the micropylar chamber is also transverse forming upper micropylar haustorial and the lower middle chambers (figure 6). The nucleus in the micropylar haustorial chamber divides without wall formation (figure 7). The micropylar haustorium develops lateral diverticulum which extends towards the raphe and starts digesting the integumentary cells (figure 8). The repeated transverse and verticular divisions in the middle chamber result in the formation of the endosperm proper (figures 6-8). The entire gamut of the ontogeny confirms 'stachys type' of endosperm development (Schnarf 1917).

3.4 Embryo development

The zygote divides transversely resulting in a terminal cell *ca* and a basal cell *cb*. The former undergoes two successive longitudinal divisions right angles to each other forming a quadrant *q* (figure 9). The quadrant later on divides transversely to form *l* and *l*¹ tiers (figure 10). The basal cell *cb* of proembryo divides transversely to form *m* and *ci* tiers (figure 10). Shortly after this *m* and *ci* divide transversely to give rise to a series of 4 superposed elements *d*, *f*, *n* and *n'* (figure 11). The further development of embryo corresponds to the *Mentha* variation of onagrad type (Johansen 1950) or period I, series A₂, Megarche type IV (Souèges 1951).

Figures 1-12. Embryology, seed coat and pericarp development in *C. umbrosa* Benth. 1. Portion of anther wall showing endothelial thickenings and bi-celled pollen grains. 2. Functional and degenerated megaspores. 3-4-Nucleated embryo sac. 4. Well organised embryo sac. 5-8. States in the endosperm development. Note: micropylar haustorial diverticulum. 9-11. Stages in embryo development. For explanation please see text. 12. Portion of L. S. of mature mericarp, showing non-mucilagenous epicarp, thin walled mesocarp and lignified endocarp.

(Ant, antipodal cell; Ch, chalazal haustorium; Cut, cuticle; Dant, degenerated antipodal cells; Dmg, degenerated megaspore; Dsyn, degenerated synergids; Eg, egg cell; En, endocarp; Ent, endothelium; Eth, endothecium; Fmg, functional megaspore; Mc, micropylar chamber; Me, mesocarp; Mh, micropylar haustorium; Mhn, micropylar haustorial nucleus; Pt, pollen tube; Sc, seed coat; Z, zygote).

In a young ovule the integument consists of 4 or 5 layers of isodiametric cells with large nuclei. After fertilization the seed coat becomes 7 or 8 layered. In a fully mature mericarp the seed coat is formed only by the outer epidermis, where the radial and inner tangential walls are sclerified (figure 12). In megaspore mother cell stage the ovary wall consists of 5 layers of homologous richly cytoplasmic cells. In the mature mericarp the cells of outer epidermal layer are non-mucilagenous. The middle layer cells are empty and thin walled. The hypodermal layer of the inner epidermis becomes highly lignified as a protective layer (figure 12).

4. Discussion

The development of anther wall corresponds to the dicotyledonous type (Davis 1966). The tapetum is secretory, dimorphic and binucleate. Cytokinesis is of simultaneous type.

Anatropous unitegmatic tenuinucellar ovule has also been reported for *Mentha* spp. (Santha Kumari 1976). Ruttle (1931, 1932) reported presence of hypostase in *Mentha* and *Lycopus* spp., degeneration of ovule in *M. requienii* and presence of 6 antipodal in *Lycopus europaeus*.

The endosperm in present taxa is cellular of stachys type according to Schnarf (1917) while it is of brunella type in *Mentha longifolia*, *Pogostemon plectranthoides* (Jaitly 1968) and in *Dysophylla quadrifolia* (Santha Kumari 1976). In *D. quadrifolia* the micropylar haustorium and chalazal haustorium are cellular and coenocytic respectively (Santha Kumari 1976). Lateral diverticulum in micropylar haustorium has also been reported by Santha Kumari (1976) in *D. quadrifolia*.

The embryo development is of onagrad type in the present taxon whereas it is of capsella type in *Mentha viridis* and *M. aquatica* (Schnarf 1931; Jaitly 1968).

The seed coat is single layered. The pericarp structure is differentiated into lignified endocarp, thin walled mesocarp and non-mucilagenous epicarp. Seed is endospermic.

References

- Davis G L 1966 *Systematic Embryology of Angiosperms* (New York: John Wiley & Sons)
- Jaitly S C 1968 Le developement de l'albumen chez les genres *Salvia*, *Hyptis*, *Ocimum*, *Pogostemon*, *Mentha* et *Leucas*; *Bull. Soc. Bot. France* 115 373-378
- Jaitly S C, Guignard J L and Mestre J C 1968 Embryogéni des Labiacées. Developement de l'embryon chez i.e. *Mentha aquatica* L; *Bull. Soc. Bot. France* 115 269-372
- Johanson D A 1950 *Plant Embryology*. Chron. Botan. Waltham, Mass, USA
- Ruttle M L 1931 Cytological and embryological studies in the genus *Mentha*; *Gartenbauwissenschaft* 4 428-468
- Ruttle M L 1932 Chromosome number, embryology and inheritance in the genus *Lycopus*; *Gartenbauwissenschaft* 7 154-174
- Santha Kumari D 1976 Endosperm development in some Labiatae (Lamiaceae); *J. Indian Bot. Soc.* 55 148-159
- Schnarf K 1917 Beitrage Zur Kenntnis der Samenentwicklung der Labiaten *Denkschar*; *Akad. Wiss. Lit. Mainz Abh Math. Naturwiss Kl.* no. 126
- Schnarf K 1931 *Vergleichende Embryologie der Angiosperm* (Berlin Strutgartl)
- Souègès R (1951) *Embryogéni et classification*, 4e fasc. Essai dum systeme embryogénique. Partie Speciale: 2c Periode du système, Paris.

Ontogeny of cambium in *Amaranthus caudatus* L. and *Achyranthes aspera* L.

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Abstract. Young seedlings have two independent vascular systems which subsequently get fused with each other. The cambium originates in the form of patches in the pericycle of the root on 6th day of its growth in *Amaranthus caudatus* and on 10th in *Achyranthes aspera*. Later on, a complete ring is formed within the pericycle which subsequently differentiates acropetally and basipetally in the hypocotyl and root. This cambium is short-lived, bidirectional and has no relation with the fascicular cambial strips of differentiating vascular bundles. Successive cambia, originated from the innermost cells of the cortex, are also bidirectional in activity and have short span of life.

Keywords. Cambium ontogeny; *Amaranthus caudatus*; *Achyranthes aspera*.

1. Introduction

Centrospermae has attracted the attention of anatomists since long due to the presence of several rows of vascular bundles of secondary origin or having alternate rows of secondary xylem and secondary phloem (see De Bary 1884; Eames and MacDaniels 1947; Matcalfe and Chalk 1950; Esau 1977). The presence of these vascular bundles or rings is usually related to the occurrence of successive cambia (Esau and Cheadle 1969; Shambie 1972; Zamski 1979). Normally a vascular cambium arises in the stem by the fusion of fascicular and interfascicular cambial strips, but in certain members of Centrospermae e.g., *Bougainvillea spectabilis*, *Mirabilis jalapa* and *Atriplex hortensis*, cambium arises in the pericyclic region and has no relation with fascicular cambial strips as it originates before the differentiation of normal vascular bundles (Stevenson and Popham 1973; Miksell and Popham 1976; Gray and Popham 1981). In view of this, developmental anatomy of seedlings and structural configuration in the internodes of the stem of *Amaranthus caudatus* L. and *Achyranthes aspera* L. have been undertaken to have a better understanding of the origin of the first and successive cambia as well as the stelar configuration of seedlings.

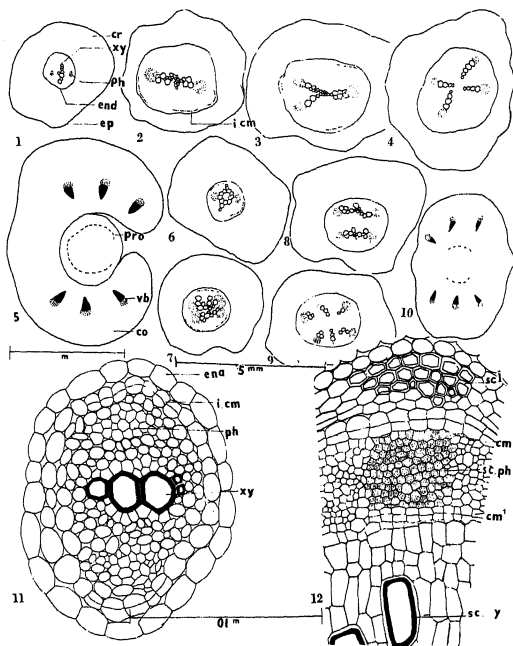
2. Materials and methods

Seeds of *A. caudatus* and *A. aspera* were procured locally. Seedlings of different ages

3. Observations

3.1 Seedling anatomy

Cross-sections of the root on 6 and 10 days old seedlings of *A. caudatus* and *A. aspera* show a diarch xylem and two radially arranged phloem patches (figures 1, 6). Pith is absent. The metaxylems of these are centrally fused (figures 1–3). In the lower



Figures 1–12. 1–5. Serial transections of a 6th day old seedling of *A. caudatus*; note the occurrence of initiating cambial patches in figures 2 and 3, 4 and 6 vascular bundles in figures 3, 4 and 5 respectively. 6–10. The same of 10th day old seedling of *A. aspera*; note the initiating cambial patches in figures 6 and 7 and enhancement of xylem elements in figure 7 and differentiation along with the adjacent of proto- and meta-xylem in figures 7 and 8 as well as 6 differentiating and fully developed bundles in figures 9 and 10. 11. Central portion of figure 1 magnified to show periclinally divided and somewhat stratified cells of initiating cambium. 12. A portion from a node of *A. aspera* depicting two successive cambial

differentiation of xylem and phloem elements (figures 4, 6). The phloem elements arrange themselves in two bilobed patches. Metaxylem and protoxylem occupies somewhat central position (figure 7). In the upper hypocotyl region two additional smaller bundles differentiate in between them and thus the number of bundles become 6 (figures 8, 9). At all the cotyledonary node 3 bundles extent into each cotyledon (figures 5, 10).

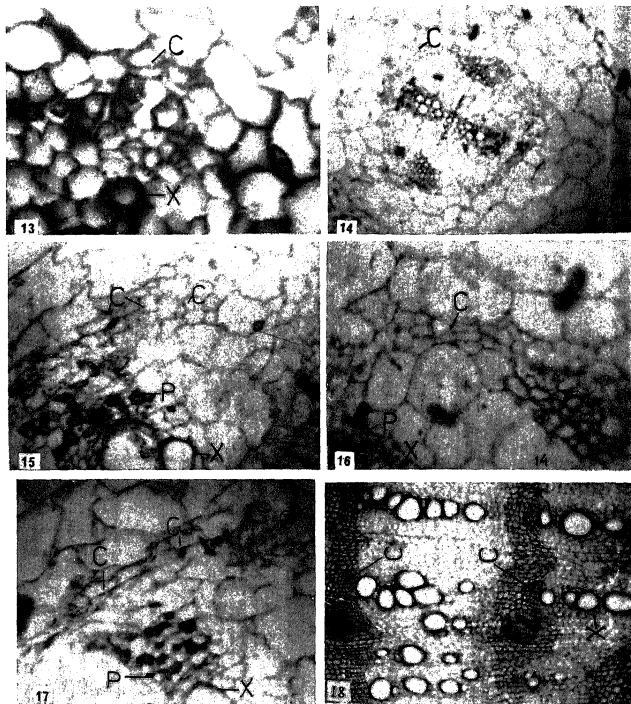
3.2 *Origin of cambium*

Seedlings of 6, 10, 14, 18 and 24 days old have been studied to locate the site of the origin of the first cambium. The cross-sections of 6 and 10 days old seedling's root of *A. caudatus* and *A. aspera* have a differentiation of the cortical and vascular region by the formation of a presumptive endodermis-like layer (figure 11). The cells of this layer are barrel-shaped, regularly arranged and have slight thickened inner tangential walls. Casparian strips develop subsequently on their radial walls. Internally this layer is followed by a layer of thin-walled smaller meristematic cells which perhaps refers pericycle due to their position. These cells divide periclinally, the derivatives radially flattened, somewhat rectangular in shape and are arranged in stratified rows (figure 11). In the beginning the initiating cambium is recognized in patches just adjacent to phloem (figures 2, 6, 7, 11, 13–16). It is absent in the upper hypocotyl region. A 10 days old seedling of *A. caudatus* and 14 days old seedling of *A. aspera* though have a cambium in roots, it is in the form of discontinuous patches in the middle region of the hypocotyl (figures 13–17). A continuous cambium in the hypocotyl has been recognized when the seedlings of *A. caudatus* and *A. aspera* have attained the age of 14 days and 24 days respectively. This cambium gives rise to some secondary parenchyma followed by secondary phloem on its outer side and secondary xylem elements along with conjunctive tissue on its inner side.

3.3 *Origin of successive cambia*

Cross-sections of young internodes have 8–10 irregularly arranged and two centrally placed medullary bundles in *A. caudatus* and *A. aspera* respectively. The pith is bound by cambium and its derivatives. This, in turn, is being followed by an endodermis, cortex and epidermis. *A. aspera* has sclerenchymatous patches in between endodermis and phloem (figure 12) and collenchymatous patches in the cortex below the ridges as the stem is quadrangular. The two medullary bundles are free in the proximal 5 or 6 internodes but are being fused in the middle of internodes to form an amphixylic strand in the rest of the distal internodes.

Later on, the cambial strips (*Amaranthus*) and the entire cambial ring (*Achyranthes*) ceased to be active. New strips or a new ring of cambium originated from the outermost cells of the secondary phloem (figure 12). The newly formed cambial strips in *Amaranthus* get themselves further differentiated inwardly and become fused with the active cambial strips of the previous cambium forming a zig-zag cambium. This process is repeated and several successive irregular or smooth cambia originate one after another. They give rise to regularly arranged bundles in *A. caudatus* and rings of secondary xylem and phloem in *A. aspera*. All the cambia happen to be bidirectional in



Figures 13–18. Cross-sections of 6th day old seedling of *A. caudatus*. 13. A part of the central region of root showing initiating cambial cells ($\times 4000$). 14. The same of 10th day old seedling from hypocotyl region showing initiating cambium ($\times 200$). 15. A part magnified to show xylem, phloem and cambium ($\times 1600$). 15–18. The same of 10th day old seedling and a young internode of *A. aspera*. 16. A part of hypocotyl region showing initiating cambium, xylem and phloem ($\times 400$). 17. A part of figure 16 magnified ($\times 1600$). 18. A part of cross-section of internode showing inactive cambial cells of successive cambia ($\times 800$). (c – cambium; p – phloem; x – xylem).

their activity. Cambial cells of inactive cambial ring can be easily recognized due to their narrow, appressed, radially arranged somewhat stratified cells in a cross-section

4. Discussion

Three points which can be profitably discussed here are: (i) the site of the first cambium; (ii) activity and origin of successive cambia; and (iii) stelar configurations of seedlings.

Regarding the origin of cambium in the members of Centrospermae there are different viewpoints. De Bary (1884) has opined that a ring of cambium develops on the outer margin of the phloem while the primary bundles are still at the stage of differentiation. Artschwager (1920) has stated that cambium patches get differentiated in the pericyclic region opposite the vascular bundles and partly as interfascicular cambial strips. Maheshwari (1930) has found cambial ring forming by the fusion of fascicular and interfascicular cambial strips of the outermost ring of vascular bundles of 3 rings present within *Boerhaavia diffusa*. Balfour (1965) has been of the opinion that in Amaranthaceae, a cambium originates in the extrafascicular region of the stem in the parenchyma cells present on the periphery of primary phloem. In *Bougainvillea spectabilis*, *Mirabilis jalapa* and *Atriplex hortensis*, wherein the ontogeny of cambium has been studied in the seedlings of various stages, the first cambium originates in the pericyclic region (Stevenson and Popham 1973; Miksell and Popham 1976; Gray and Popham 1981). Present findings regarding the ontogeny of cambium have corroborated the work of Popham and his students as the cambium has been found to be pericyclic in origin. It has no relation with the fascicular cambium of the axial bundles which differentiate inbetween the xylem and phloem. Fascicular cambial strips have limited activity as in the medullary bundles of *Boerhaavia* (Maheshwari 1930).

There are two different views regarding the origin and activity of successive cambia. According to one, the first formed cambium remains permanently active throughout the life of the plant (De Bary 1884; Wilson 1924; Boureau 1957). A modification of this view has been proposed by Balfour (1965) who maintains that the first formed cambial ring remains active as it is self perpetuating on its outer side. The secondary phloem gets differentiated within it and subsequently its outermost layers give rise to a new cambium. Her work has influenced Philipson and Ward (1965) to categorize cambium as bidirectional and unidirectional. Centrospermae according to them have unidirectional cambium as secondary xylem and secondary phloem are produced on the same side. The advocates of the second view have opined that first formed cambium has limited activity and after its cessation another cambium develops just adjacent to the secondary phloem mostly from the innermost cortical layers either as complete or partial ring or in patches which subsequently form a ring (Artschwager 1920; Maheshwari 1930; Joshi 1937). Esau and Cheadle (1969) after reinvestigating *Bougainvillea spectabilis* have reported that anomalous distribution of vascular tissue is due to origin of successive cambia one after another. This concept has been upheld by Bhambie (1972), Pannikar and Bhambie (1974), Stevenson and Popham (1973), Miksell and Popham (1976), Zamski (1979) and Gray and Popham (1981) in various taxa of Centrospermae. Present observations on the origin of successive cambia and their activity fully substantiate the idea of Esau and Cheadle (1969). Concentric rows of vascular bundles can be easily explained due to these cambia.

Regarding the fusion of medullary bundles in the distal internodes of *Achyranthes*, the present findings are in accordance with those of Joshi (1934) and Srinivasan (1966).

Maturation of xylem starts in the basipetal direction. In young seedlings, the vasculature of shoot is independent of root-hypocotyl-cotyledon. Later on, the vasculature of stem gets superimposed and fused with that of root-hypocotyl-cotyledon. The present observations thus support Thoday's (1939) interpretation, who has envisaged the double origin of vascular system in the seedlings of angiosperms. Such two independent systems have been described from a number of plants (Weaver 1960; Pillai and Sukumaran 1969; Bhambie and Nigam 1976; Gupta and Bhambie 1978; Goyal and Pillai 1979; Dubey *et al* 1981).

Acknowledgement

The authors are grateful to the Chairman of Botany Department for the facilities.

References

- Artschwager E F 1920 On the anatomy of *Chenopodium album* L.; *Am. J. Bot.* **7** 252–260
- Balfour E 1965 Anomalous secondary thickening in Chenopodiaceae, Nyctaginaceae and Amaranthaceae; *Phytomorphology* **15** 111–122
- Bhambie S and Nigam B C 1976 The anatomy of normal and tricotylous seedlings in *Trianthema triquetra* Rottler ex. Willd; *J. Indian Bot. Soc.* **55** 190–193
- Bhambie S 1972 Correlation between form, structure and habit in some lianas. *Proc. Indian Acad. Sci.* **B75** 246–256
- Boureau E 1957 *Anatomie Vegetale* (Paris: Presses Universitaires de France)
- De Bary A 1884 *Comparative anatomy of Phanerogams and Ferns*. English transl. by F O Bower and D H Scott (London: Clarendon Press)
- Dubey V P, Awasthi D K and Singhal V P 1981 Comparative anatomical observations on the dicotylous and tricotylous seedlings of *Raphanus sativus* L. (Brassicaceae); *Acta Bot. Indica* **9** 134–137
- Eames and MacDaniels 1947 *An Introduction to Plant Anatomy*, 2nd edition (New York: McGraw Hill Book Co. Inc.)
- Esau K 1977 *Anatomy of seed plants* 2nd edition (New York: John Wiley and Sons, Inc.)
- Esau K and Cheadle V I 1969 Secondary growth in *Bougainvillea*; *Ann. Bot.* **33** 807–819
- Gary L Yarrow and Popham R A 1981 The ontogeny of the primary thickening meristem of *Atriplex hortensis* L. (Chenopodiaceae); *Am. J. Bot.* **68**(8) 1042–1049
- Goyal S C and Pillai A 1979 Normal and tricotylous seedlings of *Sesamum indicum* Linn; *J. Indian Bot. Soc.* **58** 46
- Gupta M L and Bhambie S 1978 Studies on Lamiaceae. II. Anatomy of the dicotylous, tricotylous, seedlings and the node of *Ocimum sanctum* L.; *J. Indian Bot. Soc.* **57** 105–109
- Joshi A C 1934 Variations in medullary bundles of *Achyranthes aspera* L. and the original home of the species; *New Phytol.* **33** 53–57
- Joshi A C 1937 Some salient points in the evolution of the secondary vascular cylinder in Amaranthaceae and Chenopodiaceae; *Am. J. Bot.* **24** 3–9
- Maheshwari P 1930 Contribution to the morphology of *Boerhaavia diffusa*; *J. Indian Bot. Soc.* **9** 42–61
- Metcalfe C R and Chalk L 1950 *Anatomy of the dicotyledons* (Oxford: Clarendon Press)
- Miksell E and Popham R A 1976 Ontogeny and correlative relationships of the primary thickening meristem in four 'o clock plants (Nyctaginaceae) maintained under long and short photoperiods; *Am. J. Bot.* **63** 427–438
- Pannikar A O N and Bhambie S 1974 *Biology of Land Plants* (Meerut: Sarita Publ.) pp 100–109
- Philpson W R and Ward J M 1965 The ontogeny of the vascular cambium in the stems of seed plants; *Biol. Rev.* **40** 534–579
- Pillai S K and Sukumaran 1969 Histogenesis, apical meristems and anatomy of *Cyamopsis tetragonoloba*; *Phytomorphology* **19** 303–312

- Hook f; *J. Indian Bot. Soc.* **39** 309–313
- Stevenson D W and Popham R A 1973 Ontogeny of the primary thickening meristem in the seedlings of *Bougainvillea spectabilis*; *Am. J. Bot.* **60** 1–9
- Thoday D 1939 The interpretation of plant structure; *Nature (London)* **144** 571–575
- Weaver H L 1960 Vascularisation of the root-hypocotyl-cotyledon axis of *Glycine max* L; *Phytomorphology* **10** 82–86
- Wilson C L 1924 Medullary bundles in relation to the primary vascular system in Chenopodiaceae and Amaranthaceae; *Bot. Gaz.* **78** 175–199
- Zamski E 1979 The mode of secondary growth and the three-dimensional structure of phloem in *Avicennia*; *Bot. Gaz.* **140** 67–76

Cololejeunea (Pedinolejeunea) furcilibulata (Berrie et Jones) Schuster: New to Asia

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Abstract. *Cololejeunea furcilibulata* has been discovered recently from South Indian territory (Kerala and Karnataka) which extends its range of distribution from Africa (Nigeria) to Asia. The Indian population shows a wide range of habitats as the plants may be corticolous, foliicolous and rupicolous. The taxon is characterised by somewhat ovate leaf with thin-walled leaf-cells devoid of trigones, biciliate lobule having undivided basal portion (inflated lobule also present often at base), autoecious as well as paroeccious sexuality and compressed 5 plicate perianth. MS received

Keywords. *Cololejeunea (Pedinolejeunea) furcilibulata*; Hepaticae; Bryophyta.

1. Introduction

A recent survey of collection of *Cololejeuneas* from India revealed the occurrence of *Cololejeunea furcilibulata* (Berrie et Jones) Schuster which adds one more number in the Indian taxa of *Cololejeunea* known earlier from this country. *Cololejeunea* subgenus *Pedinolejeunea* to which the present species belongs is so far represented in the Indian subcontinent by 4 species viz. *C. himalayensis* (Pande et Misra) Schuster (Pande and Misra 1943a; Bonner 1977), *C. lanciloba* Steph. (Pande and Misra 1943b), *C. planissima* (Mitt.) Abeyw. (Mizutani 1961; Abeywikrama 1959; Stephani 1916) and *C. kashyapii* Udar et Srivastava (Udar and Srivastava 1985).

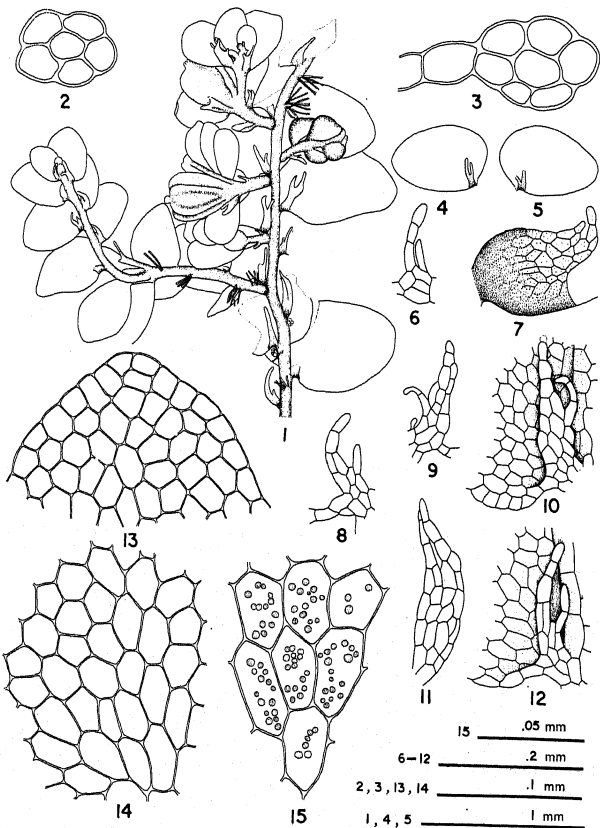
The present report of *C. furcilibulata*-a species known so far only from Nigeria where it grows epiphytic on fibrous roots of *Elasie quineensis* (see Jones 1954), constitutes a new record not only for the bryophytic flora of India but also for Asia and thus widely extending the range of distribution.

The Indian population shows a wide range of habitat preferences and interesting pattern of their vertical distribution. They grow as rupicolous and corticolous taxa at sea level in Trivandrum (Kerala) but as they migrate vertically at higher altitudes they may become either foliicolous as in Jog Falls (Karnataka: 600 m) or remain corticolous as in Agumbe (Karnataka: 791 m).

2. Taxonomic description

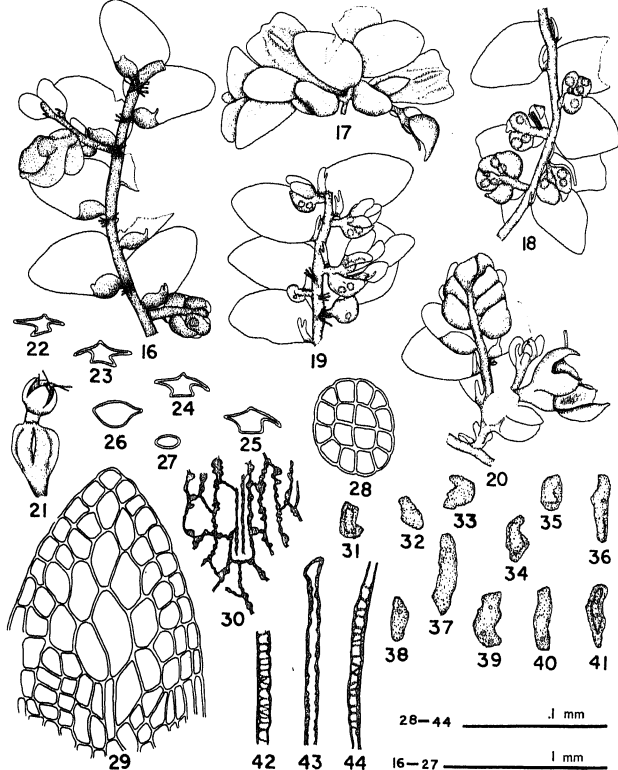
Cololejeunea furcilibulata (Berrie et Jones) Schuster, Nova Hedwigia 9: 178, 1963.
Leptocolea furcilibulata Berrie et Jones, Trans. British Bryol. Soc. 2(3): 408-438, 1954.

Plants green, appressed to the substratum. Stem usually 2-4 μ m long, 0.9-1.0 mm wide with leaves, branching irregularly pinnate, 0.06 mm across diameter with



Figures 1-15. *Cololejeunea furcilibulata* (Berrie et Jones) Schuster. 1. A plant with male and female inflorescences. 2,3. Cross-sections of the stem. 4,5. Leaves. 6-12. Leaf-lobules. 13. Marginal leaf-cells. 14. Basal leaf-cells. 15. oil-bodies.

6-cortical cells, $17 \times 17-31 \times 24 \mu\text{m}$ and one medullary cell, $20 \times 17-24 \times 20 \mu\text{m}$, ventral cells of the cortex smaller than other cortical and medullary cells. Rhizoids grouped, hyaline. Leaves imbricate, widely-obliquely spreading, according from the



Figures 16–44. *Cololejeunea furciculobulata* (Berrie et Jones) Schuster. 16–20. Plants with male and female inflorescences showing autoecious and paroeccious sexuality. 21. Perianth with dehiscent capsule. 22–27. Cross-sections of the perianth. 28. Cross-section of seta. 29. Outer layer of capsule wall. 30. Inner layer of capsule wall. 31–41. Spores. 42–44. Elaters.

or somewhat narrowed, marginal cells small, rectangular, $16\text{--}29 \times 12\text{--}21\ \mu\text{m}$, median cells pentagonal-hexagonal, $24\text{--}37 \times 20\text{--}29\ \mu\text{m}$, walls thin, trigones and intermediate nodular thickenings absent, basal cells elongated, hexagonal, $32\text{--}49 \times 20\text{--}33\ \mu\text{m}$, cuticle smooth, oil-bodies 3–15 per cell, rounded, $1\text{--}4\ \mu\text{m}$, smooth or with very faint granules; leaf-lobule variable, biciliate, ligulate or inflated, ligulate lobule

ligulate lobule and with broad and arched keel in inflated lobule, stylus unicellular, hyaline, $12 \times 8 \mu\text{m}$, gemmae not seen.

Autoecious as well as paroecious. Male inflorescence on a short lateral branch or just below the perianth; male bracts in 2–3 pairs (when autoecious) or single (when paroecious), strongly inflated with 1–2 antheridia per bract. Female inflorescence terminal on a short lateral branch, with one subfloral innovation which is again floriferous; female bracts usually in one pair, the lobe of female bract slightly smaller than the leaf-lobe, $0.41 \times 0.22 \text{ mm}$, margin entire, lobule of the lower bract bifid and of the upper entire, ligulate, $0.18 \times 0.08 \text{ mm}$. Perianth obovate, subcompressed, $0.45 \times 0.27 \text{ mm}$, 5-plicate, with a low dorsal plica and two sharp lateral and two ventral plicae. Seta articulated with 12 peripheral and 4 central cells; capsule spherical, dehiscing into 4 distinct valves; capsule wall hyaline, two layered, cells of the outer layer $16 \times 8 - 41 \times 25 \mu\text{m}$, with uniform thickening, cells of the inner layer, $24 \times 16 - 29 \times 21 \mu\text{m}$, with irregular confluent nodular thickening. Spores irregularly elongated in shape, hyaline, $24 - 49 \times 12 - 21 \mu\text{m}$, with minute spines. Elaters few, hyaline, attached to the valve, $164 \times 8 \mu\text{m}$, with sinuate, spiral or annular thickening.

2.1 *Distribution and Habitat*

2.1.1 *Type Locality*

Nigeria. *Habitat*: Corticolous, foliicolous, rupicolous. *Range*: Nigeria, India (Karnataka, Kerala).

2.2 *Specimens examined*

LWU 4755/81, Locality: Agumbe (Karnataka) alt. ca. 791 m., Habitat: Corticolous, Leg.: DK, AK and USA, May 4, 1981; LWU 6373/82, Locality: Zoo at Trivandrum (Kerala) at sea level, Habitat: Rupicolous, Leg.: R Udar and Party, September 30, 1982; LWU 6392/82, Locality: Neyyar Dam at Trivandrum (Kerala) at sea level, Habitat: Corticolous, Leg.: R Udar and Party, September 30, 1982; LWU 6508/82, Locality: Jog Falls (Karnataka) alt. ca. 600 m, Habitat: foliicolous, Leg.: R Udar and Party, September 27, 1982.

3. *Discussion*

This taxon was earlier described under the generic epithet *Leptocolea* (Jones 1954) but Schuster (1963), transferred it under *Cololejeunea* (see also Bonner 1977). However, it belongs to the subgenus *Pedinolejeunea* due to thin walled leaf-cells, variable lobule (ligulate-inflated) and apical hyaline papilla.

The name of plant itself indicates its distinctive feature – “the furcate lobule” having two cilia which may be of equal size (figure 11) or the inner cilia may be smaller, of various shape either curved away from or towards the axis (figures 6, 8–10, 12). Occasionally saccate lobules are also present which have inflated base with the upper portion either bifid or remaining undivided (figures 7, 16).

Jones (1954) described *C. furcilibulata* as monoecious from Nigeria. In the Indian

is also present. It is noteworthy that although the paroecious condition was not described for the African plants but figure 3a in Jones (1954) shows complete similarity with the Indian plants having paroecious condition (figure 17). In paroecious plants, with mature perianth, it is generally difficult to find out the presence of antheridium in male bracts but the presence of characteristic inflated leaf-lobule just below the perianth (figure 17) clearly suggests their identity with male bracts. Besides mature plants, few young plants are also represented in the collection in which the perianth has not yet developed and only archegonia are present. The antheridia are clearly seen in the axil of bracts present just below the female inflorescence, confirming the paroecious sexuality (figure 19). The perianth is 5-plicate. The serial sections of the perianth show the gradual disappearance of plicae from apex to base (figures 22–27). Mature sporophyte has spherical capsule and articulated seta with 4 central and 12 peripheral cells (figure 28). The capsule dehisces into 4 distinct valves (figure 21). The cells of the capsule wall are with hyaline thickenings not clearly seen in outer layer but inner layer cells show irregular confluent nodular thickening (figures 29, 30). The spores are irregularly shaped with minute spines. Often these spines are grouped forming rosette like structures (figures 31–41). The elaters are few and attached to capsule wall. They are hyaline with walls sinuately thickened (figure 43) but in few elaters very faint annular and spiral bands are also seen (figures 42, 44).

Acknowledgements

Grateful thanks are due to the Council of Scientific and Industrial Research, and to the Department of Science and Technology (Scientific and Engineering Research Council), New Delhi for financial assistance.

References

- Abeywikrama B A 1959 The genera of the liverworts of Ceylon; *Ceylon J. Sci. (Bio-Sci)* **2** 33–81
 Bonner C E B 1977 *Index Hepaticarum VIIa Supplementum A-C*, Germany
 Jones E W 1954 African Hepatics X. *Leptocolea* and *Cololejeunea*; *Trans Br. Bryol. Soc.* **2** 408–438
 Mizutani M 1961 A revision of Japanese Lejeuneaceae; *J. Hattori Bot. Lab.* **24** 115–302
 Pande S K and Misra R N 1943a Studies in Indian Hepaticae I—On a new species of *Leptocolea* Evans from the western Himalayas; *Proc. Nat. Acad. Sci.* **13** 25–35
 Pande S K and Misra R N 1943b Studies in Indian Hepaticae II—On the epiphyllous liverworts of India and Ceylon I; *J. Indian Bot Soc.* **22** 159–169
 *Schuster R M 1963 An annotated synopsis of the genera and sub-genera of Lejeuneaceae I. Introduction, annotated keys to subfamilies and genera; *Nova Hedwigia* **9** 1–203
 Stephani F 1916 *Species Hepaticarum* V, 1–1044 Geneve
 Udar R and Srivastava G 1985 *Cololejeunea (Pedinolejeunea) kashyapii* sp. nov. from Karnataka India; *Geophytology* **15** 64–66

* Not seen in original

Structure, distribution and taxonomic significance of trichomes in some *Indigofera* L. (Fabaceae)

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Abstract. Structure and distribution patterns of the trichomes occurring on the standard petals in 8 species of *Indigofera* L. have been investigated. In all, 4 trichome types exhibiting 7 distribution patterns have been recognised. Their taxonomic significance has been shown by providing a key for the identification of the species studied.

Keywords. Trichomes; taxonomy; *Indigofera*.

1. Introduction

Though floral trichomes have been long recognised to play a significant role in the biology of a plant such as defence, pollination, dispersal of seeds, fruits and other forms of propagules (Small 1913; Luttage 1971; Levin 1973; Heslop-Harrison and Heslop-Harrison 1975) and display much diversity. So far no sustained efforts seem to have been made in utilising their characters for taxonomic purposes. The present investigation on the standard petals was taken up to make a detailed study of the trichome structure, distribution and taxonomic value in some species of *Indigofera*.

2. Material and methods

Mature flower buds of eight species of *Indigofera* (table 1) were collected and fixed in Carnoy's fixative (Johansen 1940). Subsequently, whole mounts of the cleared standard petals (cleared with 1% KOH) were prepared, stained them with 1% aniline blue in lactophenol and mounted in glycerine (Ramayya and Rajagopal 1968). Besides whole mounts, flower buds of the species studied were also microtomed and stained following conventional methods (Johansen 1940), for observing the trichome morphology on the standard petals. Data pertinent to the trichome types, their morphology and distribution patterns are based on observation of a minimum of 10 samples. Terms used for describing the trichomes are after Ramayya (1962), Vijay Kumar (1983) and Prabhakar *et al* (1984).

3. Observations

Name of the species	Trichome type(s) and their distribution pattern(s)						
	Pattern I	Pattern II	Pattern III	Pattern IV	Pattern V	Pattern VI	Pattern VII
<i>I. aspalathoides</i> Vahl	—	—	—	—	A	—	—
<i>I. astragalina</i> DC.	—	—	A	—	—	—	—
<i>I. cordifolia</i> Heyne	—	—	—	B,C	—	—	—
<i>I. glandulosa</i> Willd.	—	—	—	—	—	—	—
var <i>glandulosa</i>	—	—	—	—	—	—	C,D
<i>I. linifolia</i> Retz.	—	C	—	—	—	B	—
<i>I. linnaei</i> Ali	—	—	—	A,B,C	—	—	—
<i>I. tinctoria</i> L.	A	—	—	—	—	—	B,C
<i>I. trifoliata</i> L.	—	—	—	—	—	—	B,C,D

A, Unicellular conical hair; B, Uniseriate macroform two-armed hair; C, Uniseriate macroform plough-shaped hair; D, Multiseriate hollow-discoid hair. I–VII, Trichome distribution patterns (for details see text).

margins in 3 taxa viz., *I. aspalathoides*, *I. astragalina* and *I. tinctoria*. The details of the trichome types and their distribution patterns in different species are as below:

3.1 Unicellular conical hair (figures 1–3)

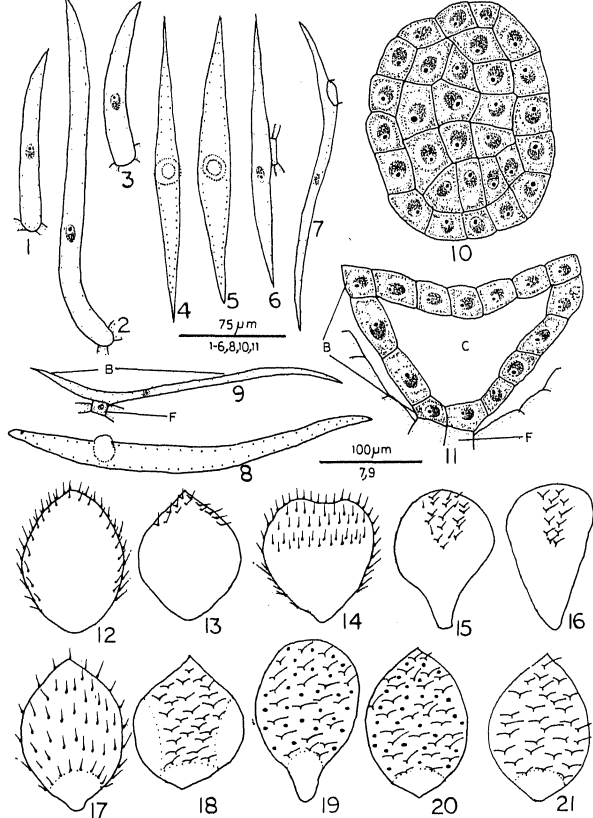
Foot: Indistinct. **Body:** Conical, tapering above, quite longer than broad; contents dense or scanty; wall thin; surface smooth or granulate. **Dist:** Common, confined to the distal half of the petal and all along the margin in *I. astragalina* (figure 14); occasional in the distal half of the petal in its midrib zone in *I. linnaei* (figure 15); common on and near margin of the petal in *I. tinctoria* (figure 12); common, all over except base of the petal, very rarely on margin in *I. aspalathoides* (figure 17).

3.2 Uniseriate macroform two-armed hair (figures 4–6)

Foot: Simple, with the base circular or oval in outline; cells broader than long; contents scanty or absent; lateral wall moderately thickened, smooth. **Body:** Unicellular, two-armed; arms equal or nearly so, 'T'-shaped, gradually tapering, pointed at both ends; contents absent; wall moderately thickened; surface smooth or punctate. **Dist.:** Common, all over except base, basilateral sides and margin of the petal in *I. linifolia* (figure 18); common, all over except base and margin of the petal in *I. trifoliata* and *I. tinctoria* (figures 20, 21); occasional in the distal half of the petal in its midrib zone in *I. linnaei* and *I. cordifolia* (figures 15, 16).

3.3 Uniseriate macroform plough-shaped hair (figures 7–9)

Trichomes similar to the one described above except, one of the arms of the body is very much reduced. **Dist.:** Common in the distal half of the petal in its midrib zone in *I. linnaei* and *I. cordifolia* (figures 15, 16); common, all over the petal except base and margin in *I. glandulosa* var *glandulosa* (figure 19), but less frequent in *I. trifoliata* and *I.*



Figures 1-21. 1-11. Trichomes of *Indigofera* L. 1-3. Unicellular conical hairs of (1) *I. astragalina*, (2) *I. linnaei* and (3) *I. tinctoria*; 4-6. Uniseriate macroform two-armed hairs (4,5) *I. linifolia* surface views; (6) *I. tinctoria* sectional view; 7-9. Uniseriate macroform plough-shaped hairs (7,8) *I. cordifolia* and *I. linnaei* sectional and surface views respectively. (9) *I. glandulosa* var. *glandulosa* sectional view. 10,11. Multiserial hollow-discoid hair of *I. trifoliata* surface and sectional views respectively. 12-21. Diagrammatic representation of standard petals of *Indigofera* L. showing trichome types and their distribution patterns (for details see text). 12. *I. tinctoria*. 13. *I. linifolia*. 14. *I. astragalina*. 15. *I. linnaei*. 16. *I. cordifolia*. 17. *I. aspalathoides*. 18. *I. linifolia*. 19. *I. glandulosa* var. *glandulosa*. 20. *I. trifoliata*. 21. *I.*

3.4 Multiseriate hollow-discoid hair (figures 10, 11)

Foot: Multicellular; cells juxtaposed; contents scanty; walls thin. **Body:** Multiseriate, shield-like, circular or oval in shape; parallel to the epidermis; cavitated in the center; cavity empty; cells slightly broader than long or vice-versa; contents dense; walls thin; surface smooth. **Dist.:** Common, all over except base and margin of petal in *I. glandulosa* var *glandulosa* and *I. trifoliata* (figures 19, 20).

4. Discussion

From the table 1, it is evident that certain trichome types occur only in particular taxa. For example, the multiseriate hollow-discoid hair is restricted to *I. glandulosa* var *glandulosa* and *I. trifoliata*, whereas *I. aspalathoides* and *I. astragalina* possess exclusively unicellular conical hairs. These species can be singled out from the others studied.

Further, the area of distribution of a trichome type on the standard petal seems to show significant variation from taxon to taxon and hence is of taxonomic significance. On the basis of a comparative study of the trichome type distribution on the standard petals in the species, in all, 7 distribution patterns have been discernable which are as follows:

Pattern I. Trichomes confined to and also near margin of petals except at its base e.g., the unicellular conical hair in *I. tinctoria* (figure 12).

Pattern II. Trichomes confined to the distal half of the petal near its margin e.g., the uniseriate macroform plough-shaped hair in *I. linifolia* (figure 13).

Pattern III. Trichomes confined to the distal half of the petal and also on its margin e.g., the unicellular conical hair in *I. astragalina* (figure 14).

Pattern IV. Trichomes confined to the distal half of the petal on its midrib zone e.g., the unicellular conical hair, uniseriate macroform two-armed hair and uniseriate macroform plough-shaped hair in *I. linnaei*; the uniseriate macroform two-armed and uniseriate macroform plough-shaped hairs in *I. cordifolia* (figures 15, 16).

Pattern V. Trichomes all over the petal including the margin except at its base e.g., the unicellular conical hair in *I. aspalathoides* (figure 17).

Pattern VI. Trichomes all over the petal except margin, base and basilateral sides e.g., the uniseriate macroform two-armed hair in *I. linifolia* (figure 18).

Pattern VII. Trichomes all over the petal except margin and base of the petal e.g., the uniseriate macroform plough-shaped hair and multiseriate hollow-discoid hairs in *I. glandulosa* var *glandulosa* (figure 19); the uniseriate macroform plough-shaped hair, uniseriate macroform two-armed hair and multiseriate hollow-discoid hairs in *I. trifoliata* (figure 20); the uniseriate macroform two-armed hair and uniseriate macroform plough-shaped hairs in *I. tinctoria* (figure 21).

Besides, the trichome distribution patterns III and V are restricted to *I. astragalina* and *I. aspalathoides* respectively, while patterns II and VI occur in one and the same species viz., *I. linifolia* (table 1). It is also of interest to note that though the pattern of trichome distribution in *I. cordifolia* and *I. linnaei* are similar (i.e. pattern IV), the latter taxon

the similarity in trichome distribution pattern in *I. glandulosa* var *glandulosa*, *I. tinctoria* and *I. trifoliata* (pattern VII), the first taxon can be separated due to the absence of uniseriate macroform two-armed hairs, while *I. tinctoria* is distinguished by the absence of multiseriate hollow-discoid hairs. Thus, these patterns are found to be of taxonomic significance. Based on the occurrence of the trichome types and their distribution patterns occurring on a given surface of the standard petal, a key for the identification of the species studied is given below:

5. Key for the identification of the *Indigofera* species:

- I. Trichomes present on the petal margin
 - II. Trichomes all over the petal except base (pattern V)
 - III. Trichomes exclusively of unicellular conical type *I. aspalathoides*
 - III. Trichomes of unicellular conical, uniseriate macroform two-armed and uniseriate macroform plough-shaped types *I. tinctoria*
 - II. Trichomes confined to the distal half of the petal (pattern III) .. *I. astragalina*
- I. Trichomes absent on petal margin
 - IV. Trichomes occur all over except base/all over except base and basilateral sides of the petal.
 - V. Trichomes occur all over except base (pattern VII)
 - VI. Uniseriate macroform two-armed hairs absent *I. glandulosa* var *glandulosa*
 - VI. Uniseriate macroform two-armed hairs present *I. trifoliata*
 - V. Trichomes occur all over except base and basilateral sides .. *I. linifolia*
 - IV. Trichomes exclusively confined to the distal midrib zone of petal (pattern IV).
 - VII. Unicellular conical hairs present *I. linmaei*
 - VII. Unicellular conical hairs absent *I. cordifolia*.

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References

- Heslop-Harrison J and Heslop-Harrison Y 1975 Enzymic removal of the proteinaceous pellicle of the stigma papilla prevents pollen tube entry in the Caryophyllaceae; *Ann. Bot.* **39** 163-165
- Johansen D A 1940 *Plant Microtechnique* (New York: McGraw-Hill Book Co Inc.)
- Levin D A 1973 The role of trichomes in plant defense; *Q. Rev. Biol.* **48** 3-15
- Luttage U 1971 Structure and function of plant glands; *Ann. Rev. Plant Physiol.* **22** 23-44
- Prabhakar M, Ramayya N and Leelavathi P 1984 Structure and distribution of the epidermal elements in the angiosperms I. Epidermal cell complex: *Geophytology* **14** 55-68

- mayya N 1962 Studies on the trichomes of some Compositae I. General structure; *Bull. Bot. Surv. India* **4** 77-88
- mayya N and Rajagopal T 1968 Foliar epidermis as taxonomic aid in "The flora of Hyderabad" I. Portulacaceae and Aizoaceae; *J. Osmania Univ. (Golden Jubilee Vol)* **4** 147-160
- all J 1913 The identification value of hair in the examination of herbs and leaves; *Pharm. J.* **36** 587-591
- ay Kumar B K 1983 *Systematics and anatomical studies of some South Indian species of Indigofera L.*, Ph.D. thesis, Osmania University, Hyderabad

Influence of combined nitrogen on growth of cyanobacteria

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Abstract. Nitrogen-fixing cyanobacteria act as fertilizer plants providing nitrogen *in situ* for rice paddies. The filamentous heterocystous cyanobacteria *Westiellopsis iyengarii* Jeeji Bai and *Mastigocladus laminosus* Cohn are potential biofertilizers in the tropical region. Results presented in this paper show that combined nitrogen does not have any beneficial effect on the growth of both *Westiellopsis iyengarii* and *Mastigocladus laminosus*. While in the former this retardation in growth is seen in spite of an increase in pigmentation, proteins and photosynthesis, in the latter, the decrease in growth appears to be a direct consequence of the lowering in the cellular content of pigments and proteins.

Keywords. Cyanobacteria; growth rate; pigmentation and oxygen evolution.

1. Introduction

Filamentous heterocystous cyanobacteria are prokaryotes endowed with the twin virtues of photosynthesis and aerobic nitrogen fixation. These nitrogen-fixing cyanobacteria act as *in situ* fertilizer plants capable of supplementing the fixed nitrogen reserves of rice paddies. A net incorporation of 20–30 kg of biologically fixed N/hectare of rice/season is attainable by adopting the algal technology developed at the Indian Agricultural Research Institute, New Delhi (Venkataraman 1972, 1981). However, this is too meagre an input to meet the N economy of the rice crop. Supplementation with nitrogenous fertilizers brings about a change in the algal flora from one predominated by nitrogen-fixing cyanobacteria to one with a preponderance of green algae (Roger *et al* 1984). Reports on the effects of combined N in laboratory cultures are controversial and include both positive (Stacey *et al* 1977) and negative (Dharmawardhene *et al* 1973; Katoh and Ohki 1975) effects. This paper deals with the effects of combined nitrogen on growth, pigmentation and photosynthesis in the filamentous, heterocystous nitrogen-fixing cyanobacteria *Mastigocladus laminosus* and *Westiellopsis iyengarii*.

2. Materials and methods

Axenic cultures of *M. laminosus* Cohn (CCAP 1447/1) and *W. iyengarii* Jeeji Bai were grown as batch cultures in N-free medium (Allen and Arnon 1955) and media amended with KNO_3 (2 and 10 mM) or NH_4Cl (2 and 10 mM). The cultures were incubated at $37 \pm 2^\circ\text{C}$ with continuous illumination of 3000 lux. Aliquots of the cultures were withdrawn at regular intervals and the filaments were harvested by centrifugation for 15 min at 3000 g. The acetone (80%) extracts of these filaments were used to monitor growth as well as their chlorophyll (Mackinney 1941) and carotenoid (Myers and Kratz 1955) contents. Growth was also measured as increase in dryweight of cyanobacterial filaments. Phycobilins were quantified from the absorption of aqueous extracts

sonicated extracts (Lowry *et al* 1951) using bovine serum albumin as standard.

3. Results

The overall pattern of growth of both cyanobacteria in the presence of combined nitrogen was similar to that of cells grown on atmospheric nitrogen (figures 1 and 2). The dry weight and log of A_{660} plotted against age of culture gave sigmoidal growth curves. Filaments of *W. iyengarii* and *M. laminosus* grown on atmospheric nitrogen showed exponential growth for atleast 16 days. In *M. laminosus*, a short lag phase (2 days) was observed only in N_2 -grown cultures. In the presence of combined nitrogen,

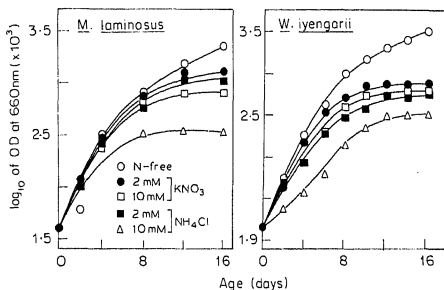


Figure 1. Growth curves of *M. laminosus* and *W. iyengarii* in the presence of combined nitrogen, based on absorbance of acetone extracts at 660 nm.

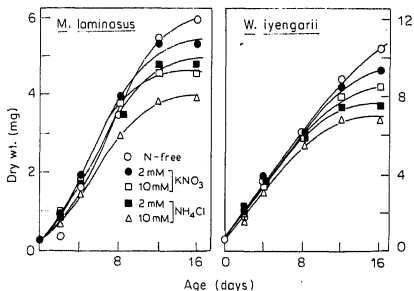
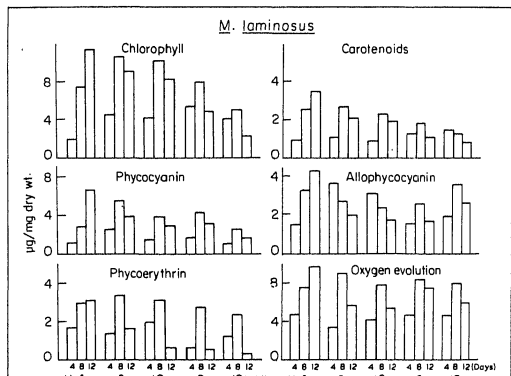


Figure 2. Growth curves of *M. laminosus* and *W. iyengarii* in the presence of combined nitrogen, based on dry weight.

growth was exponential for 10 days in *W. iyengarii* and 12 days in *M. laminosus*. In *M. laminosus* grown with 10 mM NO_3^- , the stationary phase set in on the 8th day (when growth was expressed as $\log A_{660}$) while in cultures grown with 10 mM NH_4^+ it set in as early as the 4th day. The dry weight continued to increase till the 12th day in these treatments. NO_3^- and NH_4^+ brought down the growth rate of both cyanobacteria, NH_4^+ being more effective.

Pigmentation was also altered in response to combined nitrogen. The chlorophyll content of the two organisms is shown in figures 3 and 4. In *W. iyengarii*, maximum chlorophyll production in N_2 and NO_3^- -grown cultures was on the 8th day while in NH_4^+ -grown cells it increased linearly till the 12th day. The quantity of chlorophyll produced by NO_3^- -grown cells was the same or slightly lower than that in N_2 -grown cells; while in NH_4^+ -grown cells the chlorophyll level increased by 1.5-fold. N_2 -grown cultures of *M. laminosus* showed increase in chlorophyll production till 12 days while in cultures grown with combined nitrogen, optimum production was on the 8th day. The chlorophyll content was similar in N_2 - and NO_3^- -grown cells while in NH_4^+ -grown cultures the level was 1.5–2 fold lesser. The overall pattern of carotenoid production in response to combined nitrogen was similar to chlorophyll in both organisms. The carotenoid content of *W. iyengarii* was 50% of chlorophyll and in *M. laminosus* it was about 30% of the chlorophyll level.

The phycobilin content of *W. iyengarii* was about 10 times that in *M. laminosus*. In N_2 -grown cells of *W. iyengarii*, phycocyanin (PC) was maximal on the 8th day (figure 4). In NO_3^- -grown cells, maximum PC was produced on the 4th day and this got progressively reduced with age. Consequently, cultures grown with NO_3^- were pale and unhealthy. The PC content was very high when grown on NH_4^+ , the quantity being



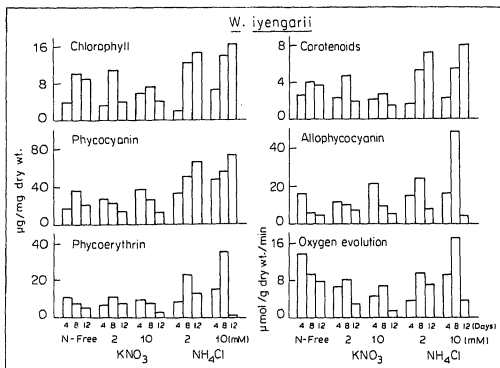
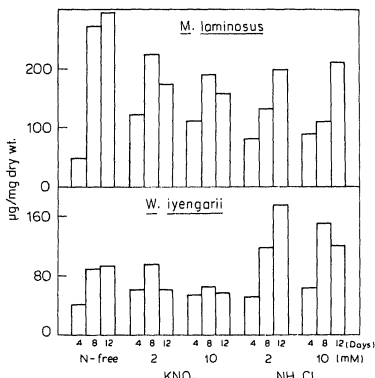


Figure 4. Changes in photosynthetic pigments and rate of oxygen evolution in *W. iyengarii* grown with combined nitrogen.



double that of N_2 -grown cells. In N_2 -grown *M. laminosus*, (figure 3) the PC content increased steadily for 12 days while in all other cultures it got reduced after the 8th day. The PC content was about 1.5-fold lower during growth on NH_4^+ . The phycoerythrin (PE) and allophycocyanin (APC) content of *M. laminosus* was nearly the same in all nitrogen sources (figure 3) and the pattern of formation was almost similar to the PC production profile. In *W. iyengarii* also, the APC and PE production was similar to PC except that their levels were nearly 3-fold higher in NH_4^+ -grown cultures (figure 4).

Photosynthetic rate measured as oxygen evolution and normalised to dry weight decreased with age in N_2 -grown *W. iyengarii* (figure 4) while it increased in N_2 -grown *M. laminosus* (figure 3). In both cyanobacteria, when grown on combined nitrogen maximum oxygen evolution was observed on the 8th day. While in *M. laminosus*, the rate of evolution was nearly the same in all cultures, in *W. iyengarii*, O_2 evolution by NH_4^+ -grown (10 mM) cultures was higher.

The protein content increased with age in N_2 -grown *W. iyengarii* (figure 5) as also in N_2 and NH_4^+ -grown *M. laminosus* (figure 5). In *M. laminosus*, the protein level was nearly the same in all cultures, while in *W. iyengarii*, NH_4^+ induced 20–50% increase in protein synthesis.

The results clearly show that combined nitrogen does not have any beneficial effect on the growth of both *W. iyengarii* and *M. laminosus*. While in the former, this reduction in growth is seen in spite of an increase in pigmentation, proteins and photosynthesis, in the latter, the reduction in growth appears to be a direct consequence of the reduction in the cellular content of pigments and proteins.

4. Discussion

Cyanobacterial incorporation in rice paddies offers a pollution-free, cost-effective, *in situ* N input. Thus, cyanobacterial supplementation, although only marginally sufficient, has an edge over chemical fertilization. Results of our study clearly show a retardation in growth of the cyanobacteria *M. laminosus* and *W. iyengarii* in the face of combined N. However, a total cessation of growth is not observed even in the presence of high concentrations of NO_3^- or NH_4^+ . Ram and Misra (1983) found that supplementation of cyanobacteria-inoculated rice fields with low levels of nitrogenous fertilizers (20 kg/ha) has a cumulative effect on the grain yield, while at higher levels of fertilization (60 kg/ha), the cyanobacterial effect is no longer evident. It is therefore obvious that the cyanobacteria can tolerate combined N to some extent and a knowledge of this upper limit for each cyanobacterium is essential. Addition of combined N in split doses which would permit the cyanobacteria to recover would also be helpful for the proper exploitation of these organisms as biofertilizers.

Acknowledgements

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References

Allen M.B. and Arnon D.I. 1955 Studies on nitrogen-fixing blue-green algae I. Growth and nitrogen fixation

- Bennett A and Bogorad L 1973 Complementary chromatic adaptation in a filamentous blue-green alga; *J. Cell. Biol.* **58** 419-435
- Dharmawardene M W N, Haystead A and Stewart W D P 1973 Glutamine synthetase of the nitrogen-fixing alga *Anabaena cylindrica*; *Arch. Microbiol.* **90** 281-295
- Kato H and Ohki K 1975 Loss of photosystem II induced by a nitrate deficiency in photoorganotrophically grown *Anabaena variabilis*; *Plant Cell Physiol.* **16** 815-828
- Lowry O H, Rosebrough N J, Farr A L and Randall R J 1951 Protein measurement with the Folin phenol reagent; *J. Biol. Chem.* **193** 265-275
- Mackinney G 1941 Absorption of light by chlorophyll solutions; *J. Biol. Chem.* **140** 315-322
- Myers J and Kratz W 1955 Relations between pigment content and photosynthetic characteristics in a blue-green alga; *J. Gen. Physiol.* **39** 11-22
- Ram G and Misra M K 1983 Effect of blue-green algae and nitrogen levels on rice yields; *IRRI Newslett.* **8** 23
- Roger P A, Remulla R and Watanabe I 1984 Effect of urea on the N₂-fixing algal flora in lowland rice at ripening stage; *IRRI Newslett.* **9** 28
- Stacey G, Van Baalen C and Tabita F R 1977 Isolation and characterisation of marine *Anabaena* sp. capable of rapid growth on molecular nitrogen; *Arch. Microbiol.* **114** 197-210
- Venkataraman G S 1972 *Algal biofertilizer and rice cultivation* (New Delhi: Today and Tomorrow Press) pp. 81
- Venkataraman G S 1981 *Blue-green algal fertilizer: A low cost technology for rice* (Rome: Food and Agriculture Organization)

Characteristics of some species of *Laccaria*, a fungal genus of significance to forestry, temperate and tropical

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Abstract. With an increasing interest in the use of selected fungi for the production of sheathing (ecto-) mycorrhizas by controlled inoculations, attempts were made to identify collections of *Laccaria* fruitbodies from woodland and forest sites in central Scotland and at high altitudes in southern India.

Although the sizes of individual fruitbodies within collections varied appreciably, it was nonetheless possible to sort the collections into 2 groups using mean sizes: one group with (i) stipes (stalks) about 7 mm long and 1 mm diam. and (ii) pilei (caps) 10 mm diam., and a second group with appreciably larger fruitbodies.

The group of larger fruitbodies was found to contain collections with either 2- or 4-spored basidia; the basidia in the group of small fruitbodies were all 2-spored.

Although of similar sizes, spores of the 4-spored specimens had different shapes as judged by the ratios (*Q* values) of spore length: spore width, separately assessed for each spore with its apiculus in view. They were either globose ($Q = 1.01$) with 8.6 spines per $9 \mu\text{m}^2$, each spine being on average $1.01 \mu\text{m}$ tall—a description conforming to *Laccaria laccata* (Scop.: Fr.) Bk. and Br- or elliptical ($Q = 1.20$) with 16.3 spines per $9 \mu\text{m}^2$, each spine being only $0.72 \mu\text{m}$ tall—a description conforming to *Laccaria proxima* (Boud.) Pat.

The spores of the group of large fruitbodies with 2-spored basidia were globose, like those of *Laccaria laccata*, but slightly larger and with more spines per unit area, 11.3 per $9 \mu\text{m}^2$, a description conforming to *Laccaria ohienensis* (Mont.) Sing. The spores of the group of small fruitbodies with 2-spored basidia were similarly globose but, in contrast, were appreciably larger, $10.1 \times 9.6 \mu\text{m}$ (compared with $6.3 \times 6.3 \mu\text{m}$ for *L. laccata*), with taller ($1.91 \mu\text{m}$) and fewer spines, 4.2 per $9 \mu\text{m}^2$, a description conforming to *Laccaria tortilis* (Bolt.) S. F. Gray.

Laccaria laccata, *Laccaria proxima* and *Laccaria tortilis* were collected in Scotland; *Laccaria proxima* and *Laccaria ohienensis* in India. Contrary to expectation *Laccaria laccata* was found less frequently than *Laccaria proxima*, the fruitbodies of both being sometimes outnumbered by those of *Laccaria tortilis* in young stands of *Betula* spp.

Keywords. *Laccaria*; *Laccaria laccata*; *Laccaria ohienensis*; *Laccaria proxima*; *Laccaria tortilis*; basidia (2- or 4-spored); *Q* values; spore echinulation.

1. Introduction

Arising from the work of Melin (1923), Bjorkman (1942), and Marx (1980), interest in the possibility of inoculating tree seedlings, and for that matter rooted-cuttings, with isolates of selected sheathing (ecto-) mycorrhizal fungi, has, in recent years, intensified. However, events in axenic ('sterile') conditions, unlike those in unsterile substrates, have indicated that the successful establishment of effective mycorrhizas on seedlings is controlled by the apposite choice of fungi, the choice being guided by the concept of 'mycorrhizal succession' (Last *et al* 1983; Mason *et al* 1983; Miller 1983). In their differing papers, mainly concerned with *Betula* spp. but now including *Picea sitchensis*

be appropriate whereas 'late-stage' fungi such as species of *Amanita*, *Russula* and *Tricholoma* would be inappropriate.

The fruitbodies (basidiomes) of *Laccaria* spp., members of the white-spored *Tricholomataceae*, have been found worldwide in a diverse array of forests with *Laccaria laccata* (Scop.: Fr.) Bk. and Br. being the species most frequently recorded. Between 1958 and 1970 *L. laccata* was found at 92 of 253 sites recorded by members of the British Mycological Society, *L. proxima* (Boud.) Pat. at 15 and *L. tortilis* (Bolt.) S. F. Gray at 5 (Rayner 1979). However, in a young plantation of *Betula pendula* Roth. and *B. pubescens* Ehrh. in central Scotland, an intensive study of *Laccaria* fruitbodies suggested that *L. laccata* might be locally uncommon (Mason *et al* 1982). Most of the many fruitbodies were attributed to the closely related 4-spored species *L. proxima* or to a rarely recorded 2-spored species *L. tortilis*. As *Laccaria* spp. appear from experiments to be leading candidates for the controlled inoculation of containerised and seedbed nursery stocks (Molina 1982; Mason *et al* 1983), it was decided to probe more deeply into the identification of relevant *Laccaria* spp. to ensure that isolates, used for inoculating young trees, were not incorrectly named as we now know has been the case in some of our work and which we suspect may have been so elsewhere. This interest was strengthened by the parallel identification of 2-, and 4-spored species of *Laccaria* in association with *Eucalyptus* spp and *Pinus patula* (Schl. and Cham.) in southern India (Natarajan 1977).

2. Methods

2.1 Collection and preservation of samples, and assessment of their macroscopic features

Fresh fruitbodies of *Laccaria* spp. were collected at (a) 9 UK sites, 8 of which were in central Scotland, where species of *Betula*, *Nothofagus* and *Quercus* were growing in association with a diverse array of other plants and (b) 4 plantations of either *Pinus patula* or *Eucalyptus* spp. near Kodaikanal (lat. 10°15'N, long. 77°31'E) in Tamil Nadu, southern India. These were augmented by dried specimens of *L. ohienensis* (Mont.) Sing. from Udhaga mandalam (Ootacamund), also in southern India.

Before being dried the macroscopic characters of fully mature fruitbodies were recorded using standard procedures and techniques. Thereafter, the samples were dried before being stored in either paper envelopes or glass bottles, containing silica gel crystals.

2.2 Assessment of microscopic details, particularly of basidiospores

Ideally, basidiospores from fresh spore prints should be examined, but to enable comparisons with all the material from India, spores, unless otherwise stated, were air-dried. They were then rehydrated in 10% ammonia before noting size and shape using a light microscope. 'Q' values, ratios of length to width, were calculated separately for each spore, always ensuring that the apiculus, of the spore being measured, was in view. Because the degree of resolution obtained with a light microscope was insufficient to obtain accurate measures of the sizes and distributions of spines, they were examined under a scanning electron microscope (SEM). For this, sections of gill were attached to aluminium stubs. They were then dried in a desiccator for a further 24 hr before being

coated with gold and examined. Photographs at magnifications ranging from $\times 1000$ to $\times 10000$ were taken and used for assessing spore characteristics.

3. Observations (table 1)

3.1 *Separation by macroscopic features of fruitbodies*

3.1.1 *Group of relatively large fruitbodies:*

(i) Subset A

Stipe (stem) 16–110 mm long and 1.5–10.0 mm wide. Characteristically fibrillose with distinct fibres running the length of the stipe which widens at the base and is covered by a white down of hyphae.

Pileus (cap) 6–47 mm diam. Convex when young but becoming flattened and often centrally depressed when old. Finely scaly when young but with a somewhat concentric pattern of dark scales on older specimens which are especially evident towards the centre of the pileus. Pileus is orange-brown when fresh, fading eventually to a light orange to yellow-brown colour. Lamellae (gills) not as darkly coloured as pileus. They are thick and wavy.

(ii) Subset B

Stipe 12–50 mm long and 2.0–5.0 mm wide. Often twisted and although slightly fibrous and tough it lacks the distinct longitudinal fibres, characteristic of Subset A. Base of stipe covered with woolly web of hyphae.

Pileus 5–33 mm. Convex when young, later becoming flattened with a central depression. Pileus has a noticeable wavy striate margin. It appears to be smoother (possibly finely scurfy) than in Subset A. It does not have dark scales. Its colour ranged from a pinkish-brown when fresh to a light orangy yellow when older and dry.

Lamellae are pinkish, thick and wavy; often dusted white with spores when mature.

(iii) Subset C

Stipe up to 45 mm long and 5 mm wide. Cylindrical solid, smooth to fibrillose, fibrils same colour as pileus.

Pileus 20–30 mm diam. Convex when young, becoming flattened and centrally depressed: margin incurved and lobed. Lamellae thick, Titian red compared with cinnamon brown of pileus.

3.1.2 *Group of relatively small fruitbodies:*

(i) Subset D

Stipe 2–18 mm long and 0.5–2.0 mm wide. Usually tapering to base; sometimes slightly fibrous.

Pileus 2–28 mm diam. Convex when young, becoming flattened and centrally depressed: margin wavy and striated. Slight scurf on surface; flesh pinkish brown.

Lamellae thick, well spaced, usually wavy and same colour as pileus.

3.2 *Separation by microscopic features (Figures 1 and 2)*

3.2.1 *Group of relatively large fruitbodies:*

(i) Subset A

Table 1. Salient characteristics of fruitbodies and spores of 4 subsets of *Laccaria* collected in association with trees in Scotland and/or Ireland

A. Fruitbody characteristics				
	Subset A (<i>L. proxima</i>)	Subset B (<i>L. laccata</i>)	Subset C (<i>L. ohienis</i>)	Subset (<i>L. torii</i>)
Pileus				
Diameter (mean)	6-47 (20) mm	5-33 (14) mm	20-30 mm	2-28 (10) mm
Texture	Dark scales prominent in centre of maturing cap	Smoothen than subset A. Flesh pink-brown when fresh	Central depression	Slight scurf on
Stipe				
Length (mean)	16-110 (40) mm	12-50 (34) mm	up to 45 mm	2-18 (7) mm
Width (mean)	1-5-10 (5) mm	2-5 (3) mm	c. 5 mm	0-5-2 (1) mm
Texture	Fibrillose with prominent axial fibres	Slightly fibrous but generally smoother than subset A	Smooth to fibrillose	Slightly fibrillose
B. Basidiospore characteristics				
Numbers of basidiospores per basidium	4	4	2	2
Shape	Elliptical	Globose to subglobose	Globose to subglobose	Globose to subglobose
Size (length \times width excluding spines)				
Light microscope	6-8-11-1 \times 6-1-9-5 μ m	7-4-10-0 \times 7-9-10-5 μ m	7-0-10-9 \times 7-0-10-1 μ m	10-3-15-2 μ m
Scanning electron microscope (mean)	6-5-7-7 (7-1) \times 5-4-6-9 (6-0) μ m	5-7-7-1 (6-3) \times 5-3-7-3 (6-3) μ m	6-5-8-3 (7-1) \times 6-3-7-8 (6-8) μ m	9-0-12-0 (10-1) \times 7-9-10-9 (9-6) μ m
Q values (from SEM observations)	1-09-1-33 (1-20)	0-92-1-11 (1-01)	1-00-1-16 (1-05)	1-00-1-14 (1-04)
Spines (measured with SEM)				
Height (mean)	0-3-1-2 (0-72) μ m	0-4-1-6 (1-01) μ m	0-5-1-4 (0-9) μ m	1-4-2-4 (1-91) μ m
Numbers per 9 μ m ²	16-3	8-6	11-3	4-2

After being prepared, spores excluding spines, only measured $6.5\text{--}7.7 \times 5.4\text{--}6.9 \mu\text{m}$ under the SEM, the decrease in size being an artefact of the preparative technique. Mean Q value—1.20 (ranging from 1.09–1.33) a value typical of ellipsoid spores.

Notwithstanding the effects on spore size, the SEM provides a means for assessing the configuration of spines, a feature of diagnostic importance for distinguishing *Laccaria* spp. Spines ranged from $0.3\text{--}1.2 \mu\text{m}$ in length with a mean of $0.72 \mu\text{m}$. There was an average of 16.3 spines per $9 \mu\text{m}^2$ of spore surface.

(ii) Subset B

Basidiospores white in mass. Under a light microscope they measured $7.4\text{--}10.0 \times 7.9\text{--}10.5 \mu\text{m}$ (excluding spines). Globose to subglobose and coarsely echinulate.

After being prepared the spores measured $5.7\text{--}6.9 \times 5.3\text{--}7.3 \mu\text{m}$ under the SEM. Mean Q value—1.01 (ranging from 0.92–1.11), values typical of globose/subglobose spores. The spines ranged from $0.4\text{--}1.6 \mu\text{m}$ in length with a mean of $1.01 \mu\text{m}$. There was an average of 8.6 spines per $9 \mu\text{m}^2$ of spore surface.

(iii) Subset C

Basidiospores white when fresh. Under a light microscope they measured $7.0\text{--}10.9 \times 7.0\text{--}10.1 \mu\text{m}$ (excluding spines). Globose to subglobose and finely echinulate.

After being prepared the spores measured $6.5\text{--}8.3 \times 6.3\text{--}7.8 \mu\text{m}$ under the SEM. The spines ranged from $0.5\text{--}1.4 \mu\text{m}$ in length with a mean of $0.9 \mu\text{m}$. Mean Q value—1.01 (ranging from 1.00–1.16), values typical of globose/subglobose spores. There was an average of 11.3 spines per $9 \mu\text{m}^2$ of spore surface.

3.2.2 Group of relatively small fruitbodies:

(i) Subset D

Basidiospores white in mass. Under a light microscope they measured $10.3\text{--}15.2 \mu\text{m}$ in diameter (excluding spines). Globose to subglobose and prominently echinulate with large robust spines.

After being prepared the spores measured $9.0\text{--}12.0 \times 7.9\text{--}10.9 \mu\text{m}$ under the SEM—these spores are significantly larger than those of Subsets A, B and C. Mean Q value—1.06 (ranging from 1.00–1.14), values typical of globose/subglobose spores. The spines ranged from $1.4\text{--}2.4 \mu\text{m}$ in length with a mean of $1.91 \mu\text{m}$. There was an average of 4.2 spines per $9 \mu\text{m}^2$ of spore surface.

4. Discussion

First and most important to forestry it is clear, from our descriptions, that many types of *Laccaria* are associated with the roots of trees. But to which species do our subsets belong?

At first sight the designation of the 2-spored forms seemed relatively straightforward with Subset C being attributable to *L. ohiensis*, and Subset D to *L. tortilis*. However, in allocating the former specific epithet it is realised that the closely related bisporic species *L. striatula* (Peck) Peck is regarded as being synonymous with *L. ohiensis* by

Table 2. Details published since 1772 of spore characteristics of *L. laccata* and *L. proxima*

Investigator	Taxonomic name	Spore characteristics		
		Size (μm)	Shape	Surface features
A. <i>Laccaria laccata</i>	Scopoli (1772)	—	—	—
	Fries (1821)	—	—	—
	Berkeley and Broome (1883)	—	—	—
	Cooke (1884)	8-9	Globose	Echinulate
	Rea (1922)	7.5-8.8 x 5.7-6.8	Globose	Spinous
	Singer (1967)	—	Sub-globose to slightly elliptical	0.3-1.2 μm
	Singer (1975)	—	—	—
	Natarajan (1977)	7.0-10.0 x 5.0-9.0	Sub-globose to elliptical	Echinulate Spines up to 1.0 μm
	Moser (1978)	7.9 x 6-7.5	Rounded to slightly elongated	Spines 0.3-1.0 μm blunt
	Lahaie (1981 pers. comm.)	7.9 x 6-8	Sub-globose to sub-elliptical	Spines 0.7-1.1 μm x 0.7-0.8 μm
	Mueller and Sundberg (1981)	6.3-11.5 x 6.3-11.7	Globose to sub-globose	Spines up to 2 μm long
B. <i>Laccaria proxima</i>	Boudier (1881)	—	Oval	Finely echinulate
	Patouillard (1887)	—	Ovoid	—
	Maire (1908)	—	—	—
	Rea (1922)	Large, 10-15 x 6-7	Elliptical	Minutely echinate
	Lange (1935)	7.5-9 x 6.75-7.5	Ovate-globose	Minutely echinate 1 μm
	Möller (1945)	7-8 x 6-7	Ovate-globose	Shortly and densely spinulose
	Singer (1967)	(10)11-12.8 x 7.5-9	Elliptical	Spines up to 2 μm long
	McNabb (1972)	8-10.5(12) x 7.5-9.5	Broadly elliptical	Spines 0.7-1.1 μm
	Moser (1978)	7.5-10(11) x 6.7-7.5	Short elliptical	Spines 1-1.5 μm
	Mueller and Sundberg (1981)	8.3-10(11) x 6.7-8.3	Oblong	Echinulate spines, less than 0

Macroscopically the 4-spored *L. proxima* should be distinguishable from *L. laccata* by the dark scales occurring on its caps (pilei) and its generally more fibrillose stipes. However, because of the extent of variation, these differences are rarely clearcut. For this reason it was found necessary, at a very early stage (in the 19th century), to take spore characteristics, particularly shape, into account (table 2). Thus Cooke (1884), when transferring Scopoli's *Agaricus laccatus* to the genus *Laccaria* was probably mindful of the definition of this genus given by Berkeley and Broome (1883)—

'Contextus tenax; hymenium cum stipite confluens; lamellae crassae sporis globosis pulverulentae'.

This description indicates that species of the genus *Laccaria* should possess globose spores. Thus, Cooke correctly included *L. laccata* and *L. tortilis* within the genus *Laccaria*. Soon afterwards, however, *Clitocybe proxima*, originally described by Boudier (1881), was transferred to his own perception of *Laccaria* by Patouillard (1887), the former referring to 'spores ovales, finement échinulées' and the latter to 'spores ovoïdes, verruqueuses'. In making this transfer Patouillard disregarded one of the features of the genus *Laccaria*—globose spores—as proposed by Berkeley and Broome (1883). Thereafter doubts seem to have arisen with an increasing lack of clarity about shape and surface echinulation, leading Singer to conclude in 1967 that it is not possible to distinguish *L. proxima* from either *L. laccata* var. *laccata* or *L. laccata* var. *proxima*, the latter having a larger fruitbody than *L. laccata* var. *laccata*, but having basidiospores in the same range of sizes. However, by combining aspects of fruitbody morphology with 3 spore characteristics we have had no difficulty in separating our collections of '4-spored' fruitbodies into 2 groups which we believe agree closely with the early intentions of Scopoli (1772), Fries (1821) and Berkeley and Broome (1883) for *L. laccata*, and of Boudier (1881) and Patouillard (1887) for *L. proxima* (table 2). Our critical spore characteristics include:

(i) *Q* value—the ratio of basidiospore length to width calculated separately for each basidiospore.

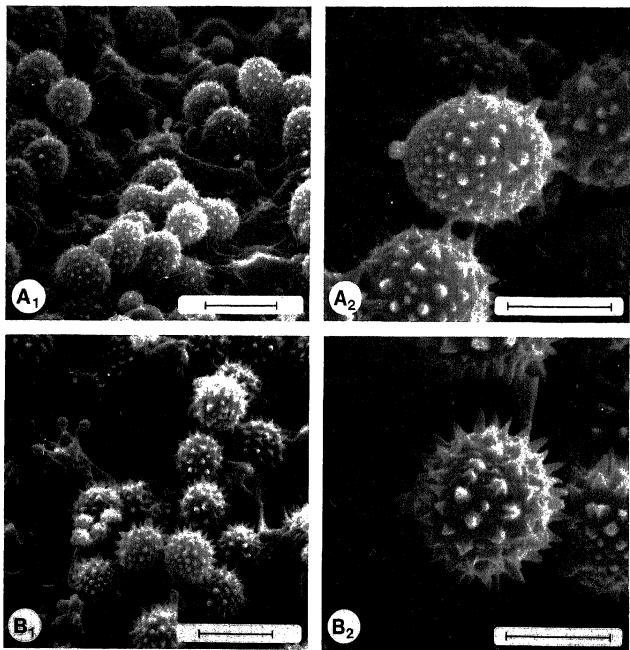
(ii) Height of spines (surface echinulation) in μm , and

(iii) Numbers of spines per unit surface area of basidiospore—for convenience numbers were counted per $9\mu\text{m}^2$.

Thus, of our 2 subsets of '4-spored' fruitbodies, 'A' generally had larger pilei than 'B'; the pilei of 'A' but not 'B' had dark scales; the stipes of 'A' unlike those of 'B' were often noticeably fibrillose; while their basidiospores were more or less of the same size, those of 'A' were elliptical (*Q* value of 1.20) in contrast to the globose spores of 'B' (*Q* value of 1.01); the spines on the basidiospores of 'B' were more prominent but less numerous than those on 'A', so making the basidiospores of 'B' appear the more echinulate. Because of these differences we consider that Subset A should be attributed to *L. proxima* and Subset B to *L. laccata*. There is, however, an undoubted need to reconsider the definitions of the different species of *Laccaria* using modern taxonomic techniques (see Malençon 1966; Mueller and Sundberg 1981; Mueller 1985). The latter, in an attempt to facilitate the identification of *Laccaria* species, included cultural characteristics in a system of numerical classification. Notwithstanding, our observations of spores of *Laccaria* spp. suggest, as when dealing with trees, that size can be highly variable and strongly influenced by environment whereas 'form' attributes, such as shape and density of surface ornamentation (spines) are strongly inherited, less strongly influenced by the environment (than size attributes) and therefore stable. Thus of the 4

Q values in the range 1.01–1.06; in contrast the spores of *proxima* are elliptical (Q value—1.20) (table 1). If further help is needed to separate the 4-spored forms, it is possible to resort to surface ornamentation—*proxima* spores having twice as many, but shorter, finer spines per unit area as *laccata* spores. Of the 2-spored forms, the basidiospores of *ohiensis* are similar to those of *laccata*, but noticeably less echinulate than those of *tortilis*, which have fewer larger spines (figures 1 and 2).

Although there are many references to *L. laccata* in the international literature dealing with mycorrhizas, we have rarely encountered this species. When summarizing the records made at 253 sites, by members of the British Mycological Society, Rayner (1979) found that *L. laccata* occurred at 92; *L. proxima* at 15 and *L. tortilis* at 5. In contrast, in our survey made of Midlothian, Scotland, *L. proxima* was found at all 8



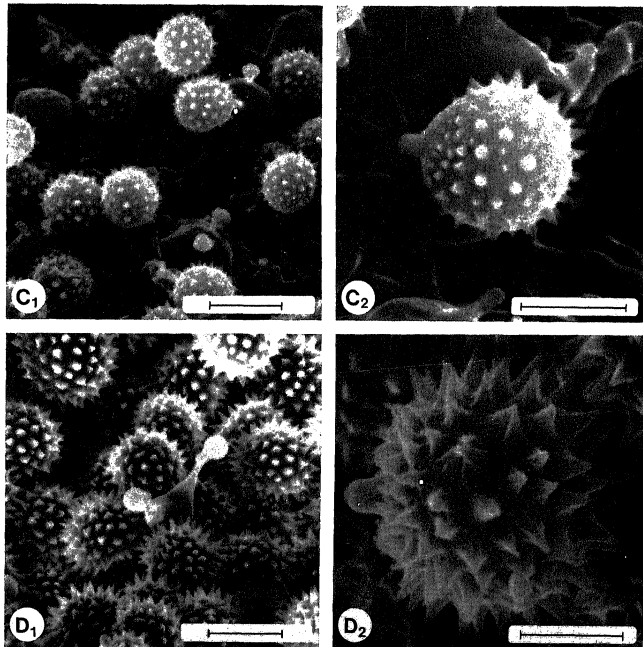


Figure 2. Scanning electron microscope photographs at two magnifications [C_1 and D_1 , $\times 1400$ (scale = $10\ \mu\text{m}$); C_2 and D_2 , $\times 4000$ (scale = $5\ \mu\text{m}$)] of basidia and basidiospores of collections of *Laccaria* with 2-spored basidia (C_1 and C_2 are typical of *L. ohienensis*; D_1 and D_2 of *L. tortilis*).

sites, *L. tortilis* at 5 and *L. laccata* at only one. Thus, in the British Mycological Society's records the ratio of *L. laccata*: *L. proxima*: *L. tortilis* was 18.4:3.0:1; in our limited survey it was 0.2:1.6:1. There are possibly many explanations for these differences, for example (i) confusion when separating *L. laccata* from *L. proxima*, and (ii) underestimates of *L. tortilis* whose small fruitbodies can be readily missed. In our study, fruitbodies of *L. tortilis* and *L. proxima* were associated with species of *Betula*, *Nothofagus* and *Quercus*. However, those of *L. tortilis* only occurred when the trees were growing in mineral soils; those of *L. proxima* occurred in peats in addition to mineral soils. Thus, in our studies with *Betula* spp. (Mason *et al* 1982), *L. proxima* has been the predominant 4-spored species but even so its fruitbodies may be greatly out-

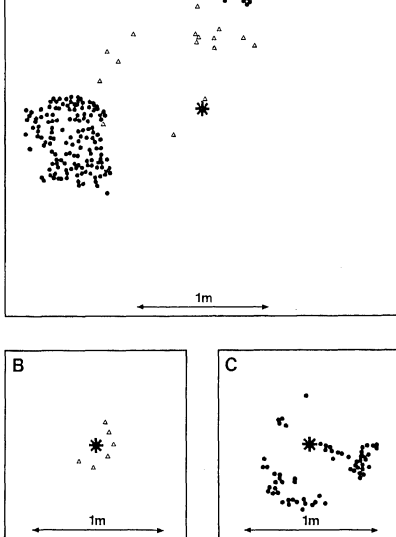


Figure 3. Spatial distribution of fruitbodies of *Laccaria tortilis* (●) and *Laccaria proxima* (Δ) that appeared around specimens of *Betula* spp. (★) (occurrences of other fungi have been omitted). **A.** configuration recorded in late summer/autumn 1977 around a *B. pendula* seedling planted into a brown earth in 1971 from seeds germinated in 1970 (Mason *et al* 1982); **B and C.** configurations recorded in late summer/autumn 1979 around clones of *B. pubescens* and *B. pendula* respectively, both having been planted into the same brown earth in November 1975 (Last *et al* 1984).

those of *L. tortilis* are strongly clumped and often arranged linearly, possibly mirroring the positions of secondarily thickened roots, the fruitbodies of *L. proxima* tend to occur in 'rings' around trees, so conforming more closely to the development of mycorrhizal fungi such as *Hebeloma crustuliniforme* (Bull. St. Amans) Quélet and *Inocybe* spp. (Mason *et al* 1982). While knowledge of the mycorrhizal capabilities of *L. laccata* and *L. proxima* has been increasing, virtually nothing is known of the activities of *L. ohiensis* and *L. tortilis*. We, and our colleagues, have recently been able to culture them on agar media—a step which should ensure their evaluation now that we suspect them of playing a possibly important role in tree biology.

References

- Berkeley M J and Broome C E 1883 Notices of British fungi; *Annals and Magazine of Natural History* V 12 370-374
- Björkman E 1942 Über die bedingungen der mykorrhizabildung bei keifer und fichte; *Symb. Bot. Ups.* 6 1-191
- Boudier E 1881 Nouvelles espèces de champignons de France; *Bull. Soc. Bot. Fr.* 28 91-98
- Cooke M C 1884 New British fungi; *Grevillea* 12 65-70
- Deacon J W, Donaldson S J and Last F T 1983 Sequences and interactions of mycorrhizal fungi on birch; *Plant Soil* 71 257-262
- Fries E 1821 *Systema Mycologicum* (E. Mauritius: Gryphiswald) 1 p. 238
- Lange J E 1935 *Flora Agaricina Danica* (Copenhagen: Soc. Adv. Mycol., Denmark) 1 p. 96
- Last F T, Mason P A and Wilson J 1983 Fine roots and sheathing mycorrhizas: their formation, function and dynamics; *Plant Soil* 71 9-21
- Last F T, Mason P A, Pelham J and Ingleby K 1984 Fruitbody production by sheathing mycorrhizal fungi: effects of 'host' genotypes and propagating soils; *For. Ecol. Manage.* 9 221-227
- McNabb R F R 1972 The Tricholomataceae of New Zealand. I *Laccaria* Berk. and Br.; *N. Z. J. Bot.* 10 461-484
- Maire R 1908 Session générale d'Octobre 1907 et notes critiques sur quelques espèces récoltées pendant la session; *Bull. Soc. Mycol. Fr.* 24 25-61
- Malençon G 1966 *Laccaria laterita* n sp, espèce thermophile; *Bull. Trimest. Soc. Mycol. Fr.* 82 181-189
- Marx D H 1980 Ectomycorrhizal fungus inoculations: a tool for improving forestation practices, in *Tropical Mycorrhiza Research* (ed.) P Mikola (Oxford: Clarendon Press)
- Mason P A, Last F T, Pelham J and Ingleby K 1982 Ecology of some fungi associated with an ageing stand of birches (*Betula pendula* and *B. pubescens*); *For. Ecol. Manage.* 4 19-39
- Mason P A, Wilson J, Last F T and Walker C 1983 The concept of succession in relation to the spread of sheathing mycorrhizal fungi on inoculated tree seedlings growing in unsterile soils; *Plant Soil* 71 247-256
- Melin E 1923 Experimentelle untersuchungen über die Birken- und Espen-mycorrhizen und ihre pilzsymbionten; *Sven. Bot. Tidskr.* 17 479-520
- Miller O K, Laursen G A and Farr D F 1982 Notes on Agaricales from Arctic Tundra in Alaska; *Mycologia* 74 576-591
- Miller O K 1983 Ectomycorrhizae in the Agaricales and Gasteromycetes; *Can. J. Bot.* 61 909-916
- Molina R 1982 Use of the ectomycorrhizal fungus *Laccaria laccata* in forestry 1. Consistency between isolates in effective colonization of containerized conifer seedlings; *Can. J. For. Res.* 12 469-473
- Möller F H 1945 *Fungi of the Faeroes I Basidiomycetes*; (Copenhagen: Munksgaard) p 295
- Moser M 1978 *Basidiomycetes II Röhrlinge und Blätterpilze; 4 Auflage* (Stuttgart: Verlag) p 535
- Mueller G M and Sundberg W J 1981 A floristic study of *Laccaria* (Agaricales) in Southern Illinois; *Nova Hedwigia Z. Kryptogamenkd.* 34 577-597
- Mueller G M 1985 Numerical taxonomic analyses on *Laccaria* (Agaricales); *Mycologia* 77 121-129
- Natarajan K 1977 South Indian Agaricales III; *Kavaka* 5 35-39
- Orton P D 1960 New check list of British agarics and boleti Part III Notes on genera and species in the list; *Trans. Br. Mycol. Soc.* 43 159-439
- Patouillard N 1887 *Les Hymenomycetes D'Europe; anatomie générale et classification des champignons superieurs*; (Paris: Librairie Paul Klincksieck) p 166
- Rayner R W 1979 The frequencies with which basidiomycete species, other than rusts and smuts, have been recorded on B. M. S. forays; *Bull. Br. Mycol. Soc.* 13 110-125
- Rea C B 1922 *British Basidiomycetae* (Cambridge: University Press) p 799
- Scopoli I A 1772 *Flora Carniolica Exhibens Plantas Carnioliae Indigenas et distributas in classes, genera, species varietates ordine Linnaeano* Tomes II; (Vindobonensis: Krauss) p 496
- Singer R 1967 Notes sur le genre *Laccaria*; *Bull. Soc. Mycol. Fr.* 83 104-123
- Singer R 1975 *The Agaricales in Modern Taxonomy* 3rd edition; (Vaduz: Cramer) p 912

Relationship of developing pods with photosynthetic characteristics of leaves in chick pea

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Abstract. Net photosynthesis rate, ribulose-1,5-bisphosphate carboxylase activity, ribulose-1,5-bisphosphate carboxylase protein and total chlorophyll content in leaves which subtend the pods vis-a-vis the pattern of pod, podwall and seed growth were analyzed in chick pea. Three sets of plants namely control, deflowered and depodded were maintained. Accompanying the initiation of pod development in control plants, there was a decline in these photosynthetic characteristics in leaves of control as well as deflowered plants. The senescence rate was higher in control plants compared to plants from which flowers or pods had been removed. The early pod growth which was mainly constituted by podwall growth, was accompanied by a higher decline in leaf net photosynthesis rate. Ribulose-1,5-bisphosphate carboxylase activity and ribulose-1,5-bisphosphate carboxylase protein decreased predominantly at the later stage of pod growth, which was mainly constituted by higher seed growth. Loss of chlorophyll was also higher at later stages of pod growth. It is suggested that both nutrient remobilization and hormonal action are probably involved during monocarpic senescence in chick pea.

Keywords. Chick pea; net photosynthesis rate; pod growth; RuBP carboxylase; senescence; subtending leaves.

1. Introduction

The onset of flowering in monocarpic plants initiates leaf senescence (Lindoo and Nooden 1976). It has been argued that the remobilization of nutrients from the leaves to the developing grains is the most plausible explanation for monocarpy (Malik and Berrie 1975; Sinclair and deWit 1976). In particular, nitrogen remobilization in crops having high nitrogen: carbon ratio in grains has been proposed to be the crucial event, leading to leaf senescence (Sinha 1977; Thomas and Stoddart 1980). However, it has also been suggested that the developing pods induce senescence of the subtending leaf by some hormonal signal, which may or may not have any relationship with the nutrient remobilization (Nooden *et al* 1978). We have attempted to characterise the senescence-associated changes in several photosynthetic components such as net photosynthesis rate, ribulose-1,5-bisphosphate carboxylase activity, ribulose-1,5-bisphosphate carboxylase protein and total chlorophyll content in subtending leaves and the parallel changes in dry matter accumulation characteristics in podwall and seeds in chick pea. Flower and pod removal treatments were practiced in order to examine the behaviour of leaf metabolic parameters in the absence of developing pods. The comparative data obtained in the present study on flowering—onwards loss of net photosynthesis rate and ribulose-1,5-bisphosphate carboxylase enzyme and accumu-

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lation of dry matter in pods indicates that leaf senescence in chick pea probably involves both nutrient remobilization and hormonal interactions.

2. Materials and methods

Leaves from the top 8 nodes of the main axis of field grown chick pea (*Cicer arietinum* L. var. JG-62; Bengal gram) were analysed throughout the present investigation (figure 1). Seeds were inoculated with proper Rhizobia culture prior to sowing. Flowering plants were tagged and in subsequent course 3 sets were maintained. In the 1st set of plants called as deflowered (Df) plants, flowers were removed at regular intervals from the specified nodes and thus pod formation was completely prevented. Pods were removed from the top 8 nodes after 10 days of flowering from the plants of 2nd set, referred to as depodded (Dp) plants. Pod formation was left undisturbed in control (C) plants. Sampling was done at flowering, young pod (10 days after flowering) and mature pod (20 days after flowering) stages.

Pods harvested from these nodes from the control plants were randomized. Dry matter accumulation in podwall and seeds was analyzed separately after drying the pods at 80°C for 48 hr. Rate of net photosynthesis was determined using intact plants by feeding $^{14}\text{CO}_2$ as described earlier (Grover *et al* 1985). The system consisted of an air tight, 4 mm thick, transparent, plexiglass chamber ($36 \times 18 \times 12 \text{ cm}^3$), connected with a battery operated air circulating pump at a speed of 1450 revolutions per min. Shoots to be fed were placed in the chamber and $^{14}\text{CO}_2$ generated by adding a few drops of 1 M HCl to $3.77 \times 10^2 \text{ KBq NaH}^{14}\text{CO}_3$ ($204 \times 10^4 \text{ KBq mol}^{-1}$) was circulated into it for 2 min. Fixed radioactivity was extracted in 80, 50 and 30% ethanol and finally in water. All extracts were combined and an aliquot was counted in a Scintillation spectrometer. Scintillation mixture contained 4 g 2,5-diphenyloxazole (PPO) and 100 mg 1,4-bis-2-(5-phenyloxazolyl)-benzene (POPOP) in 1 litre toluene.

Crude enzyme extract for assaying ribulose-1,5-bisphosphate (RuBP) carboxylase activity was prepared by the method of Marco *et al* (1979) in 0.1 M Tris-HCl buffer, pH 8.3, containing 20 mM MgCl_2 and 1 mM reduced dithiothreitol (DTT). Partial purification and activation of the enzyme was done by passing the supernatant obtained after centrifugation through a sephadex G-25 column (15 cm length \times 1.7 cm inner diameter) previously equilibrated with activation buffer (extraction buffer containing 10 mM NaHCO_3). Activity was assayed following Bjorkman (1968) with the only modification that reduced glutathione was replaced by reduced cysteine in the reaction mixture. The composition of the reaction mixture was as follows (in μmol): NaHCO_3 2.5; Cysteine 1.25; EDTA 0.1; MgCl_2 2.5 and Tris-HCl 10.0 (pH 8.3). Reaction was carried out at 25°C for 1 min using 0.2 ml of reaction mixture, 0.05 ml of enzyme extract, 0.1 ml of RuBP ($1.5 \mu\text{mol ml}^{-1}$) and 0.1 ml of $\text{NaH}^{14}\text{CO}_3$ solution ($1.88 \times 10^2 \text{ KBq ml}^{-1}$), ($104 \times 10^4 \text{ KBq mol}^{-1}$) and fixed ^{14}C activity was determined.

Soluble proteins, extracted in chilled 0.05 M Tris-HCl buffer (pH 7.5) containing 5 mM reduced cysteine and 5 mM EDTA, in 1:3 w/v ratio, were subjected to discontinuous polyacrylamide gel electrophoresis following Davis (1964). After polymerizing the gels, an aliquot containing 200 μg of the protein sample, determined by the method of Lowry *et al* (1951), was layered on the top of each gel. Electrophoresis was carried out in cold by applying 3 milli ampere current per tube. Staining was done

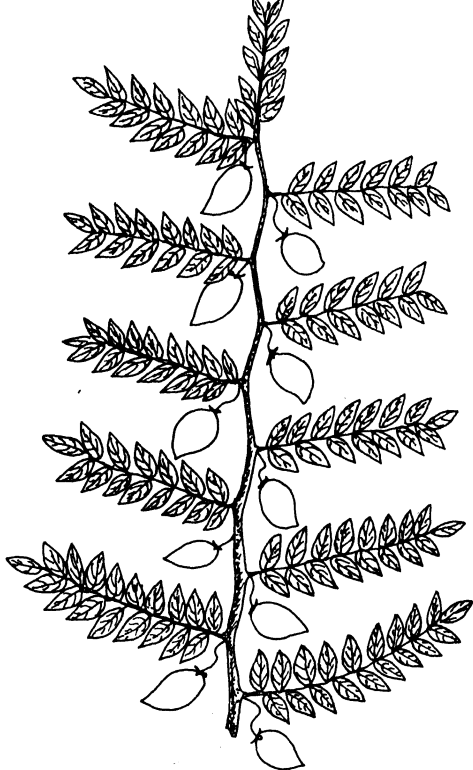


Figure 1. Apical portion of the main axis in chick pea. Top 8 nodes were used in the present work.

gels in 7 % acetic acid, gel scanning was done at 620 nm by Gilson spectrophotometer. Freshly harvested leaf tissue was extracted in chilled 80 % acetone and absorbance was read at 663 and 645 nm using spectrophotometer for determining total chlorophyll

The dry matter accumulation in pods in the phase from flowering to young pod stage (phase I) was 35% of the total pod weight at harvest (table 1). The individual contribution of podwall and seed weight was 62 and 38%, respectively. In phase II (young pod to mature pod stage), the dry matter accumulation in pods was 65% of the total pod weight out of which the individual contribution of podwall and seeds was 27 and 73% respectively. Of the total dry weight at harvest, the podwall and seeds accumulated 81 and 18%, respectively in phase I and 19 and 82%, respectively, in phase II.

Leaf net photosynthesis rate, subsequent to the flowering, declined appreciably (table 2). The extent of its loss was higher in control plants as compared to deflowered and depodded plants. RuBP carboxylase activity also showed a continuous decline with the initiation of pod growth but unlike net photosynthesis rate, the extent of loss

Table 1. Dry matter accumulation in pod, podwall and seeds in chick pea.

Stage	Component	Dry matter, mg ± SE
Young pod	Podwall	32.8 ± 1.8
	Seeds	20.0 ± 0.58
	Pods	52.8 ± 1.51
Mature pod	Podwall	40.4 ± 2.55
	Seeds	109.6 ± 6.39
	Pods	150.0 ± 8.33

Values are given on per pod basis. SE represents standard error.

Table 2. Changes in net photosynthesis rate in leaves of control, Df and Dp plants at flowering, young pod and mature pod stage in chick pea.

Stage	Treatment	Net photosynthesis rate ± SE (mg CO ₂ dm ⁻² h ⁻¹)
Flowering		31.3 ± 2.9
Young pod	Control	10.7 ± 1.0
	Deflowered	24.5 ± 0.6
Mature pod	Control	7.1 ± 1.6
	Deflowered	19.6 ± 2.6
	Depodded	8.3 ± 1.4

SE represents standard error.

control, Df and Dp plants at flowering, young pod and mature pod stage in chick pea.

Stage	Treatment	RuBP carboxylase activity \pm SE ($\mu\text{mol CO}_2 \text{ g fwt}^{-1} \text{ h}^{-1}$)
Flowering		339 \pm 12
Young pod	Control	275 \pm 2.0
	Deflowered	295 \pm 11.0
Mature pod	Control	51.6 \pm 3.7
	Deflowered	68.6 \pm 0.7
	Depodded	61.9 \pm 0.3

SE represents standard error.

in RuBP carboxylase activity was higher in phase II as compared to phase I (table 3). The leaves from Df plants showed 7 and 33% higher RuBP carboxylase activity compared to control at young and mature pod stages, respectively. As against C, leaves from depodded plants at mature pod stage showed 20% higher enzyme activity.

The densitometer tracings of the electrophoretic pattern of the soluble proteins are given in figure 2. The initial broad peak was taken as corresponding to RuBP carboxylase, based on the R_f value obtained with purified RuBP carboxylase protein in a separate run under identical conditions. By the end of phase I, the loss in peak area was 11 and 3% in C and Df treatments respectively as compared to flowering. The corresponding loss at mature pod stage was 61, 34 and 54% in C, Df and Dp treatments, respectively. Total chlorophyll content, like RuBP carboxylase activity and RuBP carboxylase protein, was reduced more in phase II compared to phase I (table 4). At young pod stage, its amount in C and Df leaves was same. However, Df leaves showed 61% more chlorophyll content than control at mature pod stage. The leaves from control plants were shed appreciably earlier than leaves from Df and Dp plants.

4. Discussion

It has often been observed in grain legumes such as pigeon pea, chick pea, mung bean and many other species that the branches or leaves having no pods in their axil remain green, while on the same plant, the leaves subtending pods turn yellow and become senescent. In chick pea, data presented in tables 2, 3 and 4 reflect that the leaves, from the axil of which either the flowers or pods were removed, exhibited relatively delayed senescence as judged by the flowering-onwards loss of net photosynthesis rate, RuBP carboxylase activity, RuBP carboxylase protein and total chlorophyll content. Taking chlorophyll level as a marker, similar conclusions have earlier been reported for soya beans (Leopold *et al* 1959; Lindoo and Nooden 1976). However, the mechanism through which the developing reproductive structures induce early senescence of the leaf which subtends, remains largely unknown. The increased remobilization of assimilates and the active transport of some hormonal signal are the

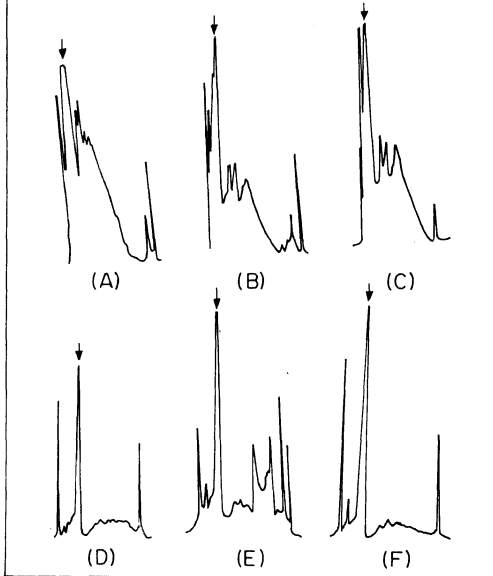


Figure 2. Densitometer traces of soluble proteins of chick pea leaves separated by discontinuous polyacrylamide gel electrophoresis. Direction of migration from left to right in each case. Arrows represent the band of RuBP carboxylase protein. **A.** Flowering stage. **B.** Young pod stage, control. **C.** Young pod stage, deflowered treatment. **D.** Mature pod stage, control. **E.** Mature pod stage, deflowered treatment. **F.** Mature pod stage, depodded treatment.

two major theories which have so far been propounded in this respect (Sesay and

ation in developing pods in chick pea revealed the initial stages and seeds at the later stages. A due to its own photosynthetic magnitude (Khanna-Chopra s, particularly for nitrogen, s due to failure in supply from phase of pod growth. The net ly phase of pod development nutrient drain, some other it role in triggering the loss of

leaves of C, Df and Dp plants at flowering, young pod and mature pod stage in chick pea.

Stage	Treatment	Total chlorophyll content \pm SE (mg g fwt ⁻¹)
Flowering		2.15 \pm 0.07
Young pod	Control	1.80 \pm 0.12
	Deflowered	1.83 \pm 0.10
Mature pod	Control	0.95 \pm 0.06
	Deflowered	1.53 \pm 0.11
	Depodded	1.09 \pm 0.02

SE represents standard error.

net photosynthesis rate in chick pea leaves. The major decline in RuBP carboxylase activity and RuBP carboxylase protein was associated with the active phase of seed growth; it is generally believed that amino-nitrogen released due to the hydrolysis of RuBP carboxylase protein is preferentially transported to developing grains (Williams and Kennedy 1978; Friedrich and Huffaker 1980).

Loss of chlorophyll content was more close to the diminution of RuBP carboxylase activity than that of net photosynthesis rate throughout senescence. The rate of net photosynthesis and RuBP carboxylase activity (*in vitro*) contributed in a differential manner towards decline of photosynthesis during leaf senescence in chick pea. To explain similar findings in barley, Friedrich and Huffaker (1980) proposed that factors such as *in vivo* regulation and stomatal aperture besides *in vitro* RuBP carboxylase activity are equally important in controlling photosynthetic rate.

The evidence in this paper suggests that the flowering-onwards loss in net photosynthesis rate on the one hand and RuBP carboxylase activity and RuBP carboxylase protein, on the other hand, in chick pea are non-parallel. Whereas the change in RuBP carboxylase protein characteristics was associated with the stage of rapid seedfill, no such association was marked for the loss in net photosynthesis rate. Thus both nutrient remobilization and hormonal action are probably involved in monocarpic senescence in chick pea. Photosynthetic assimilation has been shown to be a limiting factor in productivity of grain legumes (Hardy *et al* 1978). A closer look into the role of developing reproductive sink in triggering the decline of photosynthetic components of the subtending leaves may help in evolving strategies for further increasing yield in these crops.

References

- Arnon D I 1949 Copper enzymes in isolated chloroplasts: Polyphenoloxidase in *Beta vulgaris*; *Plant Physiol.* **24** 1–15
- Bjorkman O 1968 Carboxydismutase activity in shade adapted and sun adapted species of higher plants; *Physiol. Plant.* **21** 1–10
- Davis B J 1964 Disc electrophoresis. II. Method and application of human serum proteins; *Ann. N. Y. Acad. Sci.* **121** 404–427
- Friedrich J W and Huffaker R C 1980 Photosynthesis leaf resistance and ribulose-1,5-bisphosphate carboxylase degradation in senescing barley leaves; *Plant Physiol.* **65** 1103–1107

- Grover Anil, Koundal K R and Sinha S K 1985 Senescence of attached leaves: Regulation by developing pods; *Physiol. Plant.* **63** 87–92
- Hardy R W F, Havelka U D and Quebedeaux B 1978 The opportunity for and significance of alteration of ribulose-1,5-bisphosphate carboxylase activities; in *Photosynthetic Crop Assimilation* (eds) H W Siegelmann and G Hind (New York: Plenum Press) pp 165–178
- Khanna-Chopra Renu and Sinha S K 1980 Allantoin content and nitrogen accumulation in relation to drymatter accumulation and yield in *Cajanus cajan* cv. Prabhat; *International Workshop on Pigeon peas* ICRISAT, India, **2** 15–19
- Leopold A C, Niedergang–Kamien E and Jamick J 1959 Experimental modification of plant senescence; *Plant Physiol.* **34** 570–573
- Lindoo S and Nooden L D 1976 The interaction of fruit development and leaf senescence in 'Anoka' soyabeans; *Bot. Gaz.* **137** 218–232
- Lowry O H, Rosenbrough N J, Farr A L and Randall H J 1951 Protein measurement with the folin – phenol reagent; *J. Biol. Chem.* **193** 265–275
- Malik N S A and Berrie A M A 1975 Correlative effects of fruits and leaves in senescence of pea plants; *Planta* **124** 169–175
- Marco G D, Grego S and Tricoli D 1979 RuBP carboxylase–oxygenase in field grown wheat; *J. Exp. Bot.* **30** 951–961
- Nooden L D, Rupp D C and Derman B C 1978 Separation of seed development from monocarpic senescence in soyabeans; *Nature (London)* **271** 354–357
- Sesay A and Shibles R 1980 Mineral depletion and leaf senescence in soybeans as influenced by foliar nutrient application during seed filling; *Ann. Bot.* **45** 47–55
- Sinclair T R and deWit C T 1976 Analysis of the carbon and nitrogen limitations to soyabean yield; *Agron. J.* **68** 319–324
- Sinha S K 1977 *Food legumes: distribution adaptability and biology of yield*; FAO Plant Production and Protection, Paper 3, Rome
- Sinha S K, Khanna-Chopra R, Chatterjee S P and Abrol Y P 1978 Composition of bleeding sap in *Vigna urens*; *Physiol. Plant.* **42** 45–48
- Thomas H and Stoddart J L 1980 Leaf senescence; *Annu. Rev. Plant Physiol.* **31** 83–111
- Williams L E and Kennedy R A 1978 Photosynthetic carbon metabolism during leaf ontogeny in *Zea mays* L. Enzyme studies; *Planta* **132** 269–274

Effect of graded doses of molybdenum on the yield and uptake of molybdenum by soybean in some acid soils of Himachal Pradesh

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Abstract. A pot experiment was conducted in a green house with soybean (cultivar lee) as a test crop to study the response of applied molybdenum in some acid soils. A significant increase in grain yield and molybdenum uptake by soybean grains was observed with increasing doses of applied molybdenum. The interaction effect of soils \times molybdenum treatments on the uptake of molybdenum and grain yield was also found significant. The studies further revealed that molybdenum response could be obtained at available soil molybdenum levels ranging from 0.025-0.290 ppm. The results further revealed that in most of the soils under study the interaction of soil factors namely pH, low available and total molybdenum, and texture may be the contributing factors to yield response of soybean to applied molybdenum.

Keywords. Acid soils; total molybdenum; available molybdenum.

1. Introduction

Among the essential micronutrients, molybdenum (Mo) is required in very small quantities by plants. Molybdenum deficiency can occur in inceptisols, podzols and acid histosols (Lucas and Knezek 1972), and consequently its low availability in acid soils has been reported by a number of workers (Mulder 1954; Vinogradov 1959; Grewal *et al* 1969). Besides this, other soil constituents like the high free Fe_2O_3 content and phosphate deficiency may also depress the availability of this element to plants in acid soils.

A significant response to applied molybdenum has been reported in soybean in soils with pH 5.1 (Hagstorm and Berger 1963). Similarly, Chen *et al* (1974) also reported an increase in soybean grain yield by Mo application. An increased Mo content and uptake by soybean due to Mo application has also been reported by Hawes *et al* (1976).

The soils of Himachal Pradesh are generally acidic and it is expected that the molybdenum deficiency may be prevalent in such soils. The previous studies on molybdenum in these soils Grewal *et al* (1969) were however confined to small area giving a patchy information on the status of molybdenum in acid soils of the state. The purpose of this investigation was therefore to examine the status of total and available molybdenum and to study the effect of applied molybdenum on the yield and Mo uptake by soybean in some acid soils of Himachal Pradesh.

dose of N and P recommended for soybean (20 kg N and 80 kg P_2O_5 /ha) was given before sowing. Molybdenum application at the rate of 0(T_1), $\frac{1}{2}$ (T_2), 1(T_3), $1\frac{1}{2}$ (T_4) and 2(T_5) ounce per acre was made by thoroughly mixing the doses of sodium molybdate in each pot. The experiment consisted of 5 treatments and two replicates. Completely randomized block design was used to keep the pots in green house. Ten seeds of soybean were sown in each pot. Finally 5 healthy seedlings were allowed to grow to maturity. Moisture level was kept at about 60% of the water holding capacity throughout the experimental period by regularly applying deionized water. Oven dry weight of the grain was recorded for each pot and samples were stored for analysis.

Available molybdenum in the soil was extracted by ammonium exalate and determined colourmetrically by the method of Johnson and Arkley (1954). Grain Mo content was determined by thiocyanate orange red colour method of Johnson and Arkley (1954), after digesting the samples with nitric acid: perchloric acid: sulphuric acid (10:4:1) mixture.

3. Results and discussion

The physico-chemical characteristics of soils are given in table 1. The pH of the soil ranged between 5.3–7.1. The ammonium exalate extractable Mo was in the range of 0.025–0.290 ppm.

3.1 Grain yield

The perusal of the data in table 2 shows significant increase in grain yield with respect to various treatments, soils and their interaction. The average increase in grain yield of soybean over control was 1.15, 1.30, 1.16, 1.19 times at $\frac{1}{2}$ OZ, 1 OZ, $1\frac{1}{2}$ OZ and 2 OZ application of molybdenum per acre, respectively.

The maximum grain yield was obtained in T_3 (1 OZ Mo/acre) in soils 1 to 5, 9, 10 and 15, whereas in soils 6, 8, 11, 14 the maximum grain yield was recorded under T_2 ($\frac{1}{2}$ OZ Mo/acre). From the results it can be inferred that in most of the soils a dose of 1 ounce Mo per acre is sufficient to meet the molybdenum requirements of soybean and to get the maximum yield. Nurpur soil did not show any response upto 1 OZ molybdenum application per acre but with further increase in molybdenum dose to $1\frac{1}{2}$ OZ Mo/acre (T_4), there was non-significant increase in grain yield.

As regards the soils, the mean grain yield in different soils ranged from 5.08–13.06 g/pot. The maximum grain yield of 13.06 g/pot was found in Katrain soil (2). Different levels of applied molybdenum did not improve the grain yield in Nurpur soil (7). Although the yield differences were not significant in Nurpur soil, yet there was significant increase in soybean grain Mo concentration and its up-take (tables 3, 4). Sedberry *et al* (1973) also did not note a significant yield increase from application of Mo when the soil pH was higher than 6.2 while studying the Mo effect on soybean in 14 soils. So higher pH (7.1) may be one of the factors restricting the yield response alongwith other soil factors. In case of Bhawarna soil there was large response to

Table 1. Physico-chemical properties of soils.

Soil	Particle size distribution (%)			Textural class	Moisture percentage at 1/3 bar pressure	pH (1:2 soil: water suspension)	OC (%)	CEC me/100 g	Total Mo(ppm)	Available Mo (ppm)
	Sand	Silt	Clay							
COA Farm, Palampur	35.0	42.1	20.2	Silty loam	27.0	5.3	0.87	11.00	0.62	0.12
Bhawarna	81.2	15.0	3.0	Loamy sand	14.0	5.4	0.99	5.80	0.68	0.29
Chimbalhar	63.0	27.2	9.0	Silty loam	25.0	5.4	0.51	4.20	0.62	0.21
Bhadiarkhar	57.4	32.0	10.0	Silty loam	24.0	5.6	0.78	6.80	0.40	0.18
Dadh	62.0	27.6	9.8	Silty loam	26.0	5.6	0.81	7.28	0.28	0.16
Andretta	14.5	71.0	14.2	Silt	33.0	5.6	1.44	8.40	0.68	0.28
Shahpur	49.0	39.4	11.1	Silty loam	25.0	5.7	0.90	8.16	0.12	0.05
Arla	69.6	21.0	9.0	Loamy sand	20.0	5.7	0.87	7.36	0.18	0.09
Ahju	37.0	40.6	21.0	Silty loam	33.0	5.8	1.35	7.60	0.28	0.14
Bhatoo	63.3	28.0	8.0	Silty loam	19.0	6.0	0.72	6.50	0.48	0.13
Samloti	70.1	18.0	11.6	Loam	15.0	6.2	0.57	6.96	0.14	0.07
Gopalpur	59.1	32.1	8.3	Silty loam	28.0	6.2	1.02	7.04	0.28	0.14
Katraia	75.2	6.1	18.2	Sandy loam	21.0	6.5	1.23	8.08	0.20	0.12
Jogindernagar	84.0	8.2	7.0	Loamy sand	17.0	6.8	0.33	3.64	0.38	0.05
Nurpur	56.0	31.3	12.2	Silty loam	20.0	7.1	0.69	12.28	0.10	0.025

Soil	Treatment					Mean
	T ₁	T ₂	T ₃	T ₄	T ₅	
Jogindernagar	10.87	9.72	13.60	12.44	10.53	11.43
Katraia	11.90	12.60	14.22	13.66	12.90	13.06
Ahju	5.24	11.87	15.03	10.84	11.47	10.89
Bhadiarkhar	12.02	12.69	12.45	11.00	11.30	11.69
Andretta	10.94	11.19	12.92	8.87	10.82	10.94
Palampur (COA) Farm	4.72	7.14	4.17	4.31	4.45	5.08
Nurpur	11.45	6.88	10.47	11.66	10.92	10.21
Shahpur	6.99	11.63	9.60	10.49	9.30	9.60
Samloti	9.09	10.22	10.24	6.56	9.53	9.13
Bhawarna	8.46	12.22	14.68	12.40	12.59	12.07
Arla	11.50	13.06	11.62	12.79	9.92	11.79
Bhatoo	8.67	8.01	9.55	10.43	10.63	9.45
Chimbalhar	10.24	10.67	11.60	11.23	12.12	11.19
Dadh	11.49	12.02	11.63	10.99	11.89	11.60
Gopalpur	3.50	8.54	16.88	10.49	13.62	10.61
Mean	9.13	10.50	11.94	10.55	10.81	
C D value		(0.05)		(0.01)		
Treatments		1.12		1.47		
Soils		1.94		2.55		
Interaction (treatments × soil)		4.35		5.71		

applied Mo even with sufficient amount of available Mo (0.29 ppm). Boswell and Anderson (1969) have also reported significant increase in soybean yield on an acid soil having 0.32 ppm available Mo. In corollary Cox and Kamprath (1972) have also noted the crop response to applied Mo with soil levels upto 0.4 ppm (available Mo) in certain soils. The interaction effect of soils and treatment was also significant (table 2). However, within certain treatments the differences were non-significant. The maximum mean grain yield of 16.88 g/pot obtained in soil × T₃ combination of Gopalpur soil (15).

Parker and Harris (1962) studied the soybean response to Mo application in moderately acid soils and reported 30.9% increase in yield on Mo application. Later Hagstrom and Berger (1963) reported increase in yield of soybean and red clover by soil application of 2 lbs sodium molybdate per acre in soils of Wisconsin ranging in pH from 5.0-5.3. Sedberry *et al* (1973) reported significant increase in soybean yield from the application of Mo in II of the 22 experiments on soils with pH ranging from 4.9-6.0. The available Mo content in the soils under study was in the range of 0.025-0.290 ppm. Most of the soils had available Mo content less than 0.15 ppm (oxalate extractable Mo) the critical limit fixed by Grigg (1953b) for oxalate extractable available Mo. The results of the present study show that response to added Mo could be obtained even with available Mo content of 0.29 ppm.

The extent of increase in the yield of soybean due to the addition of Mo depends upon the content of total and available Mo in the soil, pH rates of application, method and time of application and the soil physical condition. As most of the soils used in the present study were deficient in total and available Mo, response to applied Mo could be

Table 3. Effect of molybdenum doses on Mo concentration in soybean grains

Soils	Treatments					Mean
	T ₁	T ₂	T ₃	T ₄	T ₅	
	Mo conc. (ppm)					
Jogindernagar	0.170	0.540	0.700	0.730	0.740	0.578
Katrain	0.110	0.280	0.390	0.400	0.590	0.357
Ahju	0.110	0.200	0.290	0.350	0.770	0.348
Bhadiarkhar	0.060	0.070	0.080	0.080	0.210	0.104
Andretta	0.020	0.050	0.070	0.070	0.150	0.073
Palampur (COA) Farm	0.025	0.060	0.075	0.065	0.115	0.066
Nurpur	0.090	0.450	0.740	0.690	1.030	0.603
Shahpur	0.025	0.235	0.110	0.200	0.175	0.149
Samloti	0.090	0.185	0.005	0.200	0.230	0.161
Bhawarna	0.235	0.230	0.450	0.044	0.600	0.391
Arla	0.070	0.405	0.370	0.480	0.700	0.405
Bhatoo	0.400	0.180	0.250	0.275	0.415	0.304
Chimbalhar	0.155	0.200	0.300	0.230	0.395	0.256
Dadh	0.120	0.210	0.440	0.420	0.570	0.353
Gopalpur	0.035	0.075	0.085	0.130	0.150	0.095
Mean	0.115	0.226	0.296	0.318	0.457	
C D value	(0.05)		(0.01)			
Treatments	0.039		0.051			
Soils	0.067		0.089			
Interaction (treatments × soils)	0.151		0.199			

anticipated. Further as the soils of Himachal Pradesh are generally acidic in reaction and in such soils the availability of Mo is generally low (Mulder 1954; Grewal *et al* 1969). So pH might also have contributed to yield response to applied Mo in most of the soils under study. Boswell (1980) reported that silt loam, sandy loam, or loamy sand soils with pH below 6.2 and soils inherently low in total Mo content generally respond to Mo application, which is also true in case of the soils under study.

3.2 Grain molybdenum concentration

The effect of Mo application on Mo concentration in soybean grains is given in table 3. The data show significant effect due to treatments, soils and their interaction (soil × treatment) on Mo concentration in grains. Within treatments, the maximum concentration was found with the application of the highest dose (2 OZ Mo/acre). The average increase in Mo concentration with respective increase in Mo levels was 1.96, 2.57, 2.76 and 3.97 times over the control.

As regards soils, the Mo concentration in soybean grains increased in most of the soils over control excepting the Bhatoo soil. Grain Mo concentration in case of Andretta soil increased significantly in T₃, T₄, T₅ treatments over the control (table 3). However, a significant increase in grain yield was obtained in T₃ only over the control treatment. At higher doses there was no response to applied Mo which may be attributed to higher total and available Mo content of this soil. The Mo concentration in soybean grains grown in different soils differed because of variable amounts of Mo

Soils	Treatments					Mean
	T ₁	T ₂	T ₃	T ₄	T ₅	
	Mo uptake $\mu\text{g}/\text{pot}$					
Jogindernagar	1.72	5.05	9.48	9.14	7.93	6.67
Katrain	1.36	3.59	5.49	5.55	7.62	4.72
Ahju	0.57	0.42	4.36	3.83	8.73	3.98
Bhadiarkhar	0.72	0.90	1.06	0.93	2.45	1.22
Andretta	0.23	0.53	0.96	0.63	1.60	0.79
Palampur (COA)	0.12	0.42	0.32	0.26	0.52	0.33
Nurpur	1.08	3.16	7.69	8.11	11.24	6.25
Shahpur	0.20	2.74	1.07	2.08	1.61	1.55
Samloti	0.89	1.79	0.88	1.39	2.31	1.45
Bhawarna	1.97	2.90	6.56	5.46	7.49	4.87
Arla	0.81	5.54	4.40	6.20	6.74	4.75
Bhatoo	3.41	1.46	2.47	2.88	4.38	2.92
Chimbalhar	1.62	2.10	3.43	2.57	4.83	2.91
Dadh	1.40	2.59	5.16	4.56	6.90	4.12
Gopalpur	0.13	0.72	1.41	1.42	2.11	1.16
Mean	1.08	2.39	3.65	3.67	5.10	
C D value		(0.05)		(0.01)		
Treatments		0.60		0.79		
Soils		1.04		1.37		
Interaction (treatments \times soils)		2.34		3.08		

present in these soils and due to different physico-chemical properties of the soils. With respect to soils in general, the maximum mean concentration of Mo was 0.603 ppm in Nurpur soil, which may be due to low available (0.025 ppm) and total 0.10 ppm molybdenum content of this soil resulting in greater uptake of applied molybdenum. These findings are supported by the results of Govindarajan and Gopala Rao (1964) who reported an increase in Mo content of senn-hemp by treatment with molybdenum salt. Similarly Gupta and Cutcliffe (1968) found that in green house studies, application of Mo either with seed or soil, increased the yield and Mo content of Brussels sprouts in fine sandy loam soil. The increase in Mo content in soybean seeds on Mo application has also been reported by Hawes *et al* (1976) and Gupta (1979).

3. Molybdenum uptake

The uptake of Mo by soybean grains as affected by different levels of Mo is given in table 4. The maximum Mo uptake was found at 2 OZ Mo/acre level. The average increase in Mo uptake with increasing doses of Mo was 2.21, 3.37, 3.39 and 4.72 times over control and it ranged between 0.35 and 6.67 μg Mo/pot.

In soils 1, 2, 3, 7, 8, 10, 11, 13, 14 and 15 the increasing doses of Mo in general increased Mo uptake in soybean grains significantly. The palampur soils (6) showed non-significant increase in Mo uptake by soybean grains. The interaction effect of soils \times Mo levels on Mo uptake was also found to be statistically significant (table 4). The maximum mean values of 5.85, 9.48, 9.14 and 11.24 μg Mo/pot were found

Jogindernagar soil \times T₄ and Nurpur soil \times T₅. The increased Mo concentration and consequently its uptake due to Mo application have also been reported by Gupta and Mackay (1968) and Gupta (1979) in different legume crops.

References

- Boswell F C 1980 Factors affecting the response of soybean to molybdenum application; in *Proceedings of World soybean Research conference—II*. Boulder Colorado, USA pp. 417–432
- Boswell F C and Anderson O E 1969 Effect of time of molybdenum application on soybean yield and on nitrogen, oil and molybdenum content; *Agron. J.* **61** 58–60
- Chen T T, Houng K H, Wei G T, Liu T P and Yang I F 1974 On the effect of lime and molybdenum applied in acid soils to the yield of soybean. *J. Chin. Agric. Chem.* **12** 61–71
- Cox F R and Kamprath E J 1972 Micronutrients soil tests. Micronutrients in Agriculture; *Soil. Sci. Soc. Am. Madison, USA* pp. 289–317
- Grewall J S, Bhumbra D R and Randhawa N S 1969 Available micronutrients status of Punjab, Haryana and Himachal Pradesh soils; *J. Indian Soc. Soils Sci.* **17** 27–33
- Grigg J L 1953b Determination of available Molybdenum; *New Zealand Soil News*, No 3 34–35
- Govindarajan S V and Gopala Rao H G 1964 Effect of micronutrients on crop response and quality in Mysore State; *J. Indian Soc. Soil Sci.* **12** 355–361
- Gupta U C and Cutcliffe J A 1968 Influence of phosphorus on molybdenum content of Brussels sprouts under field and green house conditions and on recovery of added molybdenum in soil; *Can. J. Soil Sci.* **48** 117–123
- Gupta U C and Mackay D C 1968 The relationship of soil properties to exchangeable, water soluble copper and molybdenum status in Podzol soils of eastern Canada; *Soil Sci. Soc. Am. Proc.* **30** 313–375
- Gupta U C 1979 Effect of methods of application and residual effect of molybdenum on the molybdenum concentration and yield of forages on podzol soils; *Can. J. Soil Sci.* **59** 183–189
- Hagstorn G R and Berger K C 1963 Molybdenum status of three Wisconsin Soils and its effect on four legume crops; *Agron. J.* **55** 399–401
- Hawes R L, Sims J L and Wells K I 1976 Molybdenum concentration of crop species as influenced by previous application of molybdenum fertilizer; *Agron. J.* **68** 217–218
- Johnson C M and Arkley T H 1954 Determination of molybdenum in plant tissue; *Anal. Chem.* **26** 572–573
- Lucas R E and Knezek B D 1972 Climate and soil conditions promoting micronutrient deficiencies in plants. Micronutrients in Agriculture; *Soil Sci. Soc. Am. Madison, USA* pp. 265–288
- Mulder E G 1954 Molybdenum in relation to growth of higher plants and microorganisms; *Plant Soil* **5** 368–415
- Parker M B and Harris H B 1962 Soybean response to molybdenum and lime and the relationship between yield and chemical composition; *Agron. J.* **54** 480–483
- Sedberry J E Jr, Dharma Putra T S, Bruphacher R H, Phillips S A, Marshall J G, Sloance K W, Melviele D R, Reham J I and Davis J H 1973 Molybdenum investigation with soybean in Louisiana. *Louisiana State Agric. Exp. Stn. Bull.* 670
- Vinogradov A P 1959 *The Geochemistry of rare and dispersed chemical elements in soils*, (Consultants Br. New York) (Translated from Russian)

Zonation in the shoot apex of *Matthiola incana* R.Br.

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Abstract. Zonation in the shoot apex of *Matthiola incana* was studied histochemically from the mature embryo to flowering. The plumular apex of the mature embryo shows a single-layered tunica covering a homogeneous mass of cells. The first evidence of zonation is seen in the shoot apex of the 2-day old seedling. Zonation is well established on the fourth day after seed wetting with a single-layered tunica covering a lightly stained central mother cell zone, a subjacent pith meristem and a densely stained peripheral zone. In the transitional apex zonation is less marked and there is increased stratification. At 6-weeks, the apex changes into the indeterminate inflorescence apex showing acropetally developing floral primordia. The axially located cells of the inflorescence apex are lightly stained. The histological and histochemical data indicate that zonation resembling that in the vegetative apex is retained to a lesser degree in the inflorescence apex as well.

Keywords. Zonation; transitional apex; inflorescence apex; histochemistry.

1. Introduction

The shoot apex in Crucifers has been studied by some investigators—*Arabidopsis thaliana*, *Capsella bursapastoris* (Vaughan 1955), *Alyssum maritimum* (Lance-Nougarède 1961), *Sinapis alba* (Bernier 1962), *Cherianthus cheiri* (Hagemann 1963) and *Brassica campestris* (Chakravarti 1953; Orr 1978). These are limited to studies on changes from the vegetative to the inflorescence apex and do not involve data ranging from the plumular to the flower. The present study fills the gap for *Matthiola incana* R.Br.

2. Materials and methods

Seeds of *Matthiola incana* R.Br. were germinated in petriplates lined with moist filter paper. Shoot apices were fixed at 24 hr intervals for the first 7 days after seed wetting and then at weekly intervals till flowering from seedlings raised in pots. Materials were fixed in FAA and Carnoy's-B, processed through TBA series, embedded in paraffin wax and sectioned at 7 μ m. Samples fixed in FAA and stained with safranin-light green with combination of tannic acid-ferric chloride (Johansen 1940) were used for anatomical studies. Measurements of height and width of shoot apices were taken from median longitudinal sections. Width was taken at the level of attachment of the youngest visible leaf primordium from the adaxial side and height from the tip of the apical dome to this basal reference point. About 15–20 replicate apices at each stage were examined and measured. The staining procedures used for various cellular components are as follows:

RNA-pyronin-Y as a stain (Tepper and Girford 1962) with perchloric acid serving as control (Erickson *et al* 1949).

(ii) DNA-Feulgen technique (Feulgen and Rossenbeck 1924) with perchloric acid as control.

(iii) Total proteins-mercuric bromophenol blue method (Mazia *et al* 1953) with acetylation (Jensen 1962) serving as control.

All photographs have been taken with a C Z Ergaval microscope using light green filter for easy comparison.

3. Observations

3.1 The plumular apex

The plumular apex refers to the apex of the mature embryo. The apex is flat with a mean diameter of $63.2\text{ }\mu\text{m}$, and is flanked by the cotyledons. It shows a single tunica layer covering a homogeneous corpus. The cells of the tunica and corpus are uniform in size and staining reaction (figure 1).

3.2 The zonate apex

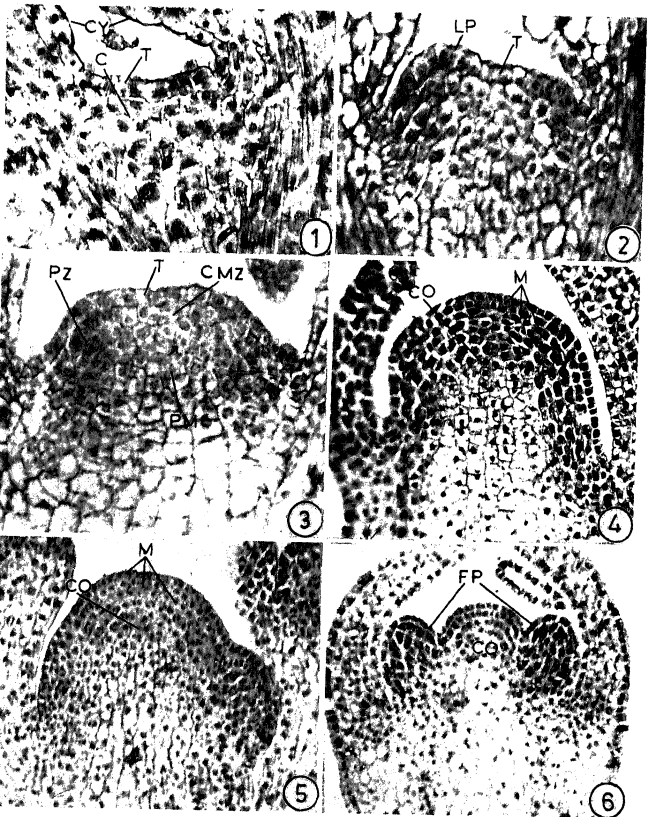
The inception of zonation is noted in the 2-day old seedling shoot apex as denser staining of the cells on the flanks in comparison with the axially located cells of the corpus (figure 2). The vegetative apices from the 4-day old seedling onwards show a well-established cytohistological zonation with a lightly stained central mother cell zone (CMZ) a densely stained peripheral zone (PZ) and a pith meristem (PM). A single tunica layer covers the shoot apex (figure 3).

The vegetative apex is a low or high dome depending on the age and plastochronic stage. The gradual age-related increase in size of the apex is maintained from germination till 5 weeks as the mean height increases from $30.0\text{--}69.2\text{ }\mu\text{m}$ and the mean diameter from $76.4\text{--}160.0\text{ }\mu\text{m}$.

The vegetative apex has a single-layered tunica having a few lightly stained axial cells. The CMZ is immediately proximal to the tunica cells at the summit of the apical dome. It shows a group of lightly stained and irregularly arranged cells. The peripheral zone forms a cylinder of 3-4 cell layers around the central mother cell zone and pith meristem. The cells are darkly stained and show anticlinal and periclinal divisions resulting in regular cell files which broaden proximally and form the site for initiation of lateral primordia. Planes of division in the CMZ indicate contribution of cells to the PZ and PM (figures 3, 12 see arrows).

3.3 The transitional apex

At 5 weeks the vegetative apex changes into the broad transitional apex having a mean height and width of $140.0\text{ }\mu\text{m}$ and $188.0\text{ }\mu\text{m}$ respectively. Zonation becomes faint and there is increased stratification of the surface layers resulting from the regular arrangement of the distal cells of the CMZ. This results in a mantle-core organisation having 3-4 darkly stained mantle layers covering a lightly stained core (figure 4). In the mantle there is a marginal difference in the depth of staining between the axially located and peripherally located cells, the former being lighter.



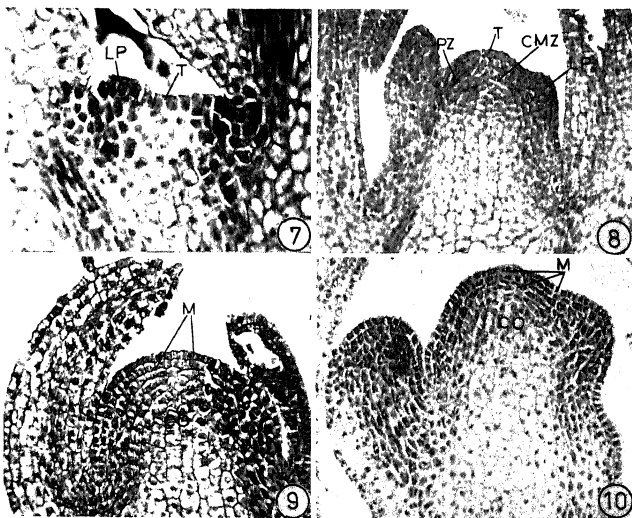
Figures 1-6. Median longitudinal sections of the shoot apex stained with safranin and light green. 1. Plumular apex without zonation, $\times 350$. 2. Two-day old seedling shoot apex showing lighter stained axially located cells, $\times 400$. 3. Three-week old vegetative apex showing well established cytohistological zonation, $\times 350$. 4. Five week old transition apex showing mantle core organisation with lightly stained axial cells, $\times 400$. 5. Seven-week old inflorescence apex showing lighter axial mantle cells, $\times 350$. 6. The flower apex, $\times 350$. Arrow indicates division figure.

mantle-core organisation. The axial cells of the mantle are lightly stained as compared to the peripheral ones. The inflorescence apex remains active till it shrivels up at about 15 weeks.

The flower apex shows a mantle-core organisation with a 2-3 layered mantle of darkly stained cells. The primordia of the parts of the flower are initiated by divisions in the mantle layers on the flanks and ultimately the whole flower apex is involved in the formation of flower parts. Cytohistological zonation is not evident (figure 6).

3.5 Histochemistry

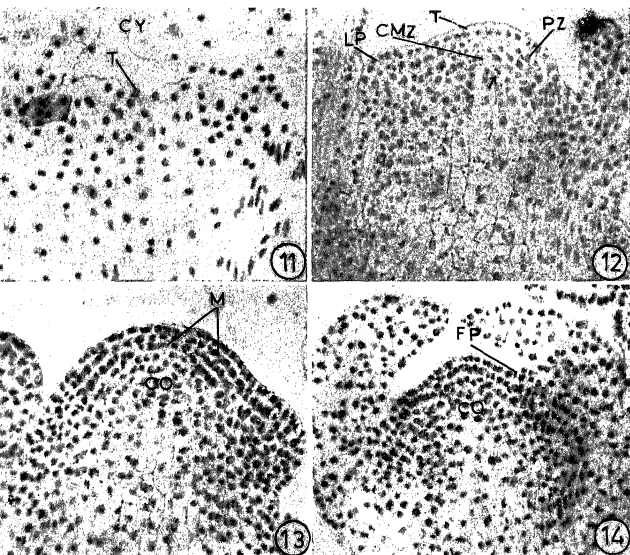
Distribution patterns of RNA, DNA and total proteins were studied for all the developmental stages. The patterns generally follow the anatomical data.



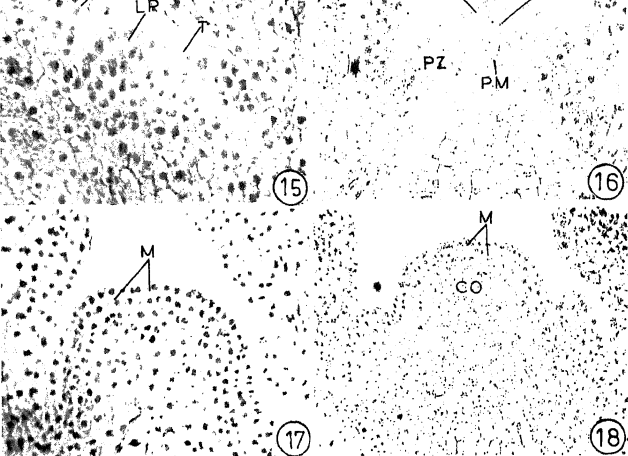
Figures 7-10. Median longitudinal sections of the shoot apex stained for RNA. 7. The seedling shoot apex, $\times 400$. 8. The vegetative apex, $\times 400$. 9. The transitional apex, $\times 400$. 10. The inflorescence apex, $\times 400$. Arrow indicates division figure.

stained (figure 7). The peripheral zone of the vegetative apex (2-4 weeks) shows more nuclear as well as cytoplasmic staining for RNA as compared with the axially located CMZ (figure 8). In the transitional and inflorescence apices the axially located cells of the mantle layers are lightly stained as compared with the peripheral mantle. The core shows lighter staining (figures 9, 10).

3.5.2 DNA: DNA also presents a more or less similar distribution pattern. Nuclei of the peripheral cell layers are darkly stained in the 2-day old apex (figure 11). The vegetative apex shows clear zonation with faintly stained nuclei in the axial tunica cells and in the CMZ and darker ones in the peripheral zone (figure 12). In the transitional apex this difference is marginal. In the inflorescence apex the cells of the peripheral mantle layers evince darker nuclei (figure 13). The nuclei in the mantle layers of the flower apex are uniformly darkly stained as compared with those in the core and the loci for primordia of the parts of the flower show darker nuclei (figure 14).



Figures 11-14. Median longitudinal sections of the shoot apex stained for DNA. 11. The seedling shoot apex, $\times 400$. 12. The vegetative apex, $\times 400$. 13. The inflorescence apex, $\times 400$. 14. The flower apex, $\times 500$. Arrow indicates division figure.



Figures 15-18. Median longitudinal sections of the shoot apex stained for total protein. 15. The seedling shoot apex, $\times 400$. 16. The vegetative apex, $\times 400$. 17. The transitional apex, $\times 400$. 18. The inflorescence apex, $\times 400$. Arrow indicates division figure.

3.5.3 Total proteins: Staining with bromophenol-blue for total proteins shows a distribution pattern similar to that of RNA. The 2-day old seedling shoot apex has a lighter stained group of axially located cells (figure 15) and the vegetative apex demonstrates clear zonation with a lighter central mother cell zone and darker peripheral zone (figure 16). The difference in the depth of staining between the CMZ and PZ becomes very faint in the transitional apex (figure 17) and more marked in the inflorescence apex (figure 18). The flower apex has a uniformly stained mantle and a lighter core.

4. Discussion

The present study shows an age-related increase in size of the shoot apex as has been reported by others (Rogan and Smith 1974; Mauseth 1978a, b; Goyal *et al* 1980). In the data presented this is followed by a gradual decrease, probably due to the rapid formation of floret primordia.

The occurrence of cytohistological zones superimposed on a tunica corpus organisation has become generally accepted in the angiosperm shoot apex. But there are few reports which trace the origin, development and establishment of zonation. The data presented here support Mauseth's (1978a) conclusion that initiation of zonation in

plastochronic cycles and that the peripheral zone is the first zone to get established. Contribution of cells to the peripheral zone and pith meristem from the CMZ supports the suggestion that the CMZ be considered as the source of cellular structure of the shoot. The CMZ as described here forms part of the continuing meristematic residue, which has been described by Newman (1965) as a source of cellular structure.

The results of histochemical localisation of RNA, DNA and total proteins follow a distribution pattern closely resembling the cytohistological zonation. These are in general agreement with previous reports (Corson and Gifford 1969; Molder and Owens 1972; Kavathekar and Pillai 1979; Goyal *et al* 1983).

The variations in apical zonation during transition from the vegetative to the reproductive stage in crucifers seem controversial. Zonation in the transitional and the inflorescence apices is less marked than in the vegetative apex as reported here. There are some crucifers without evidence of any transitional phase (*A. maritimum*, Lance-Nougarède 1961; *A. thaliana*, Vaughan 1955), whereas in some others a transitional stage is reported (*S. alba*, Bernier 1962; *B. campestris*, Orr 1978). Orr (1978) in *B. campestris* reported complete restoration of cytohistochemical zonation in the inflorescence apex.

The present data also show that the vegetative and inflorescence apices are anatomically different because of the added stratification in the latter. The transitional and inflorescence apices show indeterminate growth because of the racemose inflorescence and this may be the reason for the maintenance of zonation in them. The peripheral mantle continues to produce flower primordia. The flower apex shows determinate growth and hence the absence of zonation. It seems that the presence or absence of zonation in this instance depends upon the type of growth of the meristem.

Acknowledgements

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References

- Bernier G 1962 Evolution of the apical meristem of *Sinapis alba* L. (Long-day plant) in long days, in short days and during the transfer from short days to long days; *Caryologia* **15** 303-325
- Chakravarti S C 1953 Organization of shoot apex during ontogeny of *Brassica campestris* L.; *Nature (London)* **171** 223-224
- Corson G E and Gifford E M Jr 1969 Histochemical studies of the shoot apex of *Datura stramonium* during transition to flowering; *Phytomorphology* **19** 189-196
- Erickson R O, Sax K B and Ogur M 1949 Perchloric acid in the cytochemistry of pentose nucleic acid; *Science* **110** 472-473
- Feulgen R and Rossenbeck K H 1924 Mikroskopisch chemischer Nachweis einer Nucleinsäure Vom Typus der Thymonucleinsäure Und die darauf beruhende Selektiv färbung Von Zellkernen in Mikroskopischen Präparaten; *Z. Phys. Chem.* **135** 203-248
- Goyal S C, Jain S and Pillai A 1983 Localisation and semi-quantitative estimation of DNA, RNA and Proteins in the shoot apex of *Papaver somniferum* L.; *Beitr. Biol. Pflanz.* **58** 67-75
- Goyal S C, Kavathekar K Y, Satija S and Pillai A 1980 Developmental anatomy of some oil-yielding plants III. The seedling shoot apex; *Proc. Indian Acad. Sci.* **B89** 7-13

- Jensen W A 1962 *Botanical histochemistry* (San Francisco: W H Freeman and Co)
- Johansen D A 1940 *Plant microtechnique* (New York: McGraw Hill Co)
- Kavathekar K Y and Pillai A 1979 Studies on the developmental anatomy of Ranales. VII. Histochemical studies of the shoot apex of *Delphinium ajacis* Linn. during transition to flowering: *Proc. Indian Nat. Acad. Sci.* **B45** 577-585
- Lance-Nougardé A 1961 Comparaison du fonctionnement reproducteur chez deux plantes vivaces construisant des inflorescences en grappe indéfinie Sans fleur terminale, *Teucrium scopodonia* L. (Labiées) et *Alyssum maritimum* Lamk (Crucifères); *C.R. Acad. Sci. (Paris)* **252** 924-926
- Mauseth J D 1978a An investigation of the morphogenetic mechanisms which control the development of zonation in seedling shoot apical meristems; *Am. J. Bot.* **65** 158-167
- Mauseth J D 1978b An investigation of the phylogenetic and ontogenetic variability of shoot apical meristems in the Cactaceae; *Am. J. Bot.* **65** 326-333
- Mazia D, Brewer P A and Alfert M 1953 The cytochemical staining and measurements of protein with mercuric bromophenol blue; *Biol. Bull.* **104** 57
- Molder M and Owens J N 1972 Ontogeny and histochemistry of the vegetative apex of *Cosmos bipinnatus* var "Sensation"; *Can. J. Bot.* **50** 1171-1184
- Newman I V 1965 Pattern in the meristems of the vascular plants. III. Persuing the patterns in the apical meristem where no cell is a permanent cell; *J. Linn. Soc. London (Bot.)* **59** 185-214
- Orr A R 1978 Inflorescence development in *Brassica campestris* L.; *Am. J. Bot.* **65** 466-470
- Rogan P G and Smith D L 1974 The development of the shoot apex of *Agropyron repens* (L.) Beauv.; *Ann. Bot.* **38** 967-976
- Tepper H B and Gifford E M Jr 1962 Detection of ribonucleic acid (RNA) with pyronin stain; *Stain Tech.* **37** 52
- *Vaughan J G 1955 The morphology and growth of the vegetative and reproductive apices of *Arabidopsis thaliana* (L.) Heynh. *Capsella bursapastoris* (L.) Medic. and *Anagallis arvensis* L.; *J. Linn. Soc. London (Bot.)* **55** 279-301

Abbreviations: C, corpus; CMZ, central mother cell zone; CO, core; CY, cotyledon; FP, primordium of the parts of the flower; LP, leaf primordium; M, mantle; PM, pith meristem; PZ, peripheral zone; T. tunica.

* Not seen in original.

Structure and cytochemistry of the pistil in *Arachis hypogaea*

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Abstract. In *Arachis hypogaea* (Papilionoideae, Leguminosae), the stigma is of the dry papillate type. The papillae are multicellular and multiseriate. They are covered with a thin lining of pellicle which responds for proteins, non-specific esterases and acid phosphatases. The style is 3–6 cm long and hollow throughout its length. The stylar canal is bordered by a layer of canal cells. The canal cells in most of the stylar region are not glandular; they are vacuolate with scant cytoplasm. The canal cells at the base of the style, however, are glandular with dense cytoplasm and prominent nuclei. The structural features of the pistil of *Arachis* are discussed with those of other Papilionoideae.

Keywords. *Arachis hypogaea*; groundnut; legume; pistil; pollen-pistil interaction; stigma; style.

1. Introduction

Studies on the details of the pistil and of pollen-pistil interaction are important not only in understanding the biology of sexual reproduction but also in applied aspects of fruit- and seed-set (see Shivanna and Johri 1985). Such basic data are also useful in manipulating hybridization barriers. Studies on these aspects on members of Leguminosae (Papilionoideae) have been initiated only recently and are, so far, confined to *Vigna* (Ghosh and Shivanna 1982), *Trifolium* (Heslop-Harrison Y and Heslop-Harrison J 1982; Heslop-Harrison J and Heslop-Harrison Y 1982), *Cicer* (Malti and Shivanna 1983), *Crotalaria* (Malti and Shivanna 1984), *Vicia* (Lord and Heslop-Harrison 1984) and *Phaseolus* (Heslop-Harrison J and Heslop-Harrison Y 1984).

Arachis hypogaea is one of the most important legumes in India. Although intensive studies are being carried out on interspecific hybridization (Sastri and Moss 1982; see also Sastri 1984) in this taxon, no details are available on the structure of the pistil and on pollen-pistil interaction. This paper reports anatomical and cytochemical details of the pistil in *Arachis hypogaea*.

2. Material and methods

Plants of *Arachis hypogaea* Linn. var. TMV-2 (an annual, branched, prostrate type), grown under field conditions were used in our studies. For cytochemical studies on the stigma, flower buds at 3 developmental stages—2 days before anthesis, 1 day before anthesis and on the day of anthesis—were used. Stigma surface proteins were localized with 0.25% coomassie brilliant blue R in 7% acetic acid (Heslop-Harrison *et al* 1974). Non-specific esterases were localized using α -naphthyl acetate as a substrate in a coupling reaction with fast blue B (Mattsson *et al* 1974) and acid phosphatases with α -naphthyl acid phosphate as a substrate in a coupling reaction with fast garnet GBC

Anatomical studies of pistils were carried out using semithin sections (2 μ m). As the pistil in *Arachis* is long (3–6 cm) about 5 mm segments from each of the following 4 levels were used for fixation and sectioning: (i) the stigma and a few millimeter of the subjacent style, (ii) middle region of the staminal tube, (iii) middle region of the calyx tube, and (iv) the lower most part of the style together with the upper part of the ovary.

Pistils were fixed in 10 % aqueous acrolein for 24 hr at 0°C and dehydrated through a 2-methoxyethanol—ethanol—*n*-propanol—*n*-butanol series (Feder and O'Brien 1968). The dehydrated material was infiltrated and embedded in JB₄ resin (Polysciences, USA). Sections were cut at 2 μ m thickness and stained with 0.1 % toluidine blue (Merck) in 0.1 M acetate buffer, pH 4.5 (Feder and O'Brien 1968); 0.25 % coomassie brilliant blue R (Weber and Osborn 1975) for proteins, periodic acid-Schiff's reagent (PAS, McGuckin and McKenzie 1958; Feder and O'Brien 1968) for insoluble polysaccharides; and 0.02 % aqueous auramine 0 (Heslop-Harrison 1977) for cuticle.

3. Observations

The flower is subsessile. The calyx forms a long tube, called the hypanthium, enclosing the ovary and most of the proximal part of the style, and appears very much like a pedicel (figure 1A, B). There are 10 stamens of which 8 bear fertile and 2 sterile anthers. Of the 8 fertile anthers, 4 are linear and the remaining 4 round, arranged alternately in a ring. Anther filaments arise from the rim of the calyx tube. The lower parts of anther filaments are fused forming a sheath around the style, above the calyx tube, while the terminal parts of the filaments are free.

The pistil is 3–6 cm long and most of it is formed by the style. The ovary is small and inconspicuous at the base of the style. About two thirds of the style is enclosed by calyx tube. The style is curved at right angles to the long axis at the tip of the calyx tube and again at the tip of the staminal tube (figure 1B). The stigma is terminal and club shaped (figure 1C). Non-receptive hairs are present on the upper part of the style.

The stigma is of the dry, papillate type (Heslop-Harrison and Shivanna 1977) without any visible exudate. The papillae are globose, multicellular and multiseriate (figure 1F). The papillae show a thin lining of proteinaceous pellicle which could be localized following staining with coomassie blue (figure 1D). The pellicle occurs on the surface of the papillae as a continuous layer. The pellicle is present in all the stages of buds studied; however, there is an increase in the intensity of staining with the maturity of the pistil.

The pellicle also responds for non-specific esterases and acid phosphatases. While esterases form a smooth and continuous layer (figure 1E) phosphatases are distributed in the form of granules. The activity of both the enzymes increases with the maturity of pistils.

The stigma surface as well as the contents of the stigmatic papillae stain intensely with sudan black B. Small globules of lipoidal material are also present in the papillae. The cuticle of the papillae is extremely thin and shows very faint fluorescence with auramine 0.

The stigma is solid with a core of transmitting tissue. The style is hollow throughout its length and is traversed by a canal which is continuous with the ovarian cavity. The

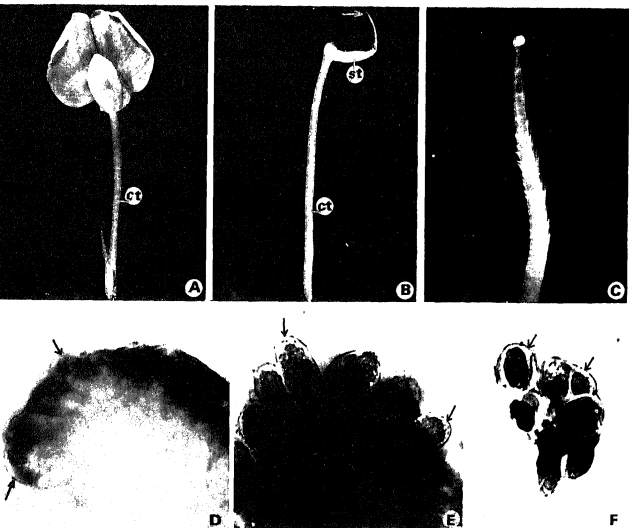


Figure 1. A. Front view of a flower soon after anthesis. Observe long calyx tube (ct) enclosing the lower part of the style. B. Entire pistil from a flower on the day of anthesis. Ovary and most of the proximal part of the style are enclosed in calyx tube (ct) and staminal tube (st). Arrow points to the stigma. C. Stigma and a part of the subjacent style at higher magnification. Stylar hairs and club shaped stigma are obvious. D. Wholmount of the stigma stained with coomassie blue. Coomassie blue positive pellicle is clearly visible at many places (arrows). E. A part of the stigma following localization of non-specific esterases. Apart from cytoplasmic esterases, pellicle of the papillae also show esterase activity (arrows). F. A few papillae from E to show multicellular nature of the papillae and pellicle (arrows).

stigmatic papillae converge at the base of the stigma and form a strand of transmitting tissue. Transections of the stigma at the base show an epidermal layer and cells of the transmitting tissue with large intercellular spaces. There is no cavity at this level (figure 2A).

Transections below the stigma show the initiation of a small stylar cavity which is not bordered by a well-demarcated layer of canal cells (figure 2B). The stylar cavity gradually widens toward the lower part of the style (figures 2C–F) and gets surrounded by a well-demarcated layer of cells, canal cells (figures 2E, F). The cortical region becomes distinguishable. The stylar cavity is not bordered by cuticle as revealed by

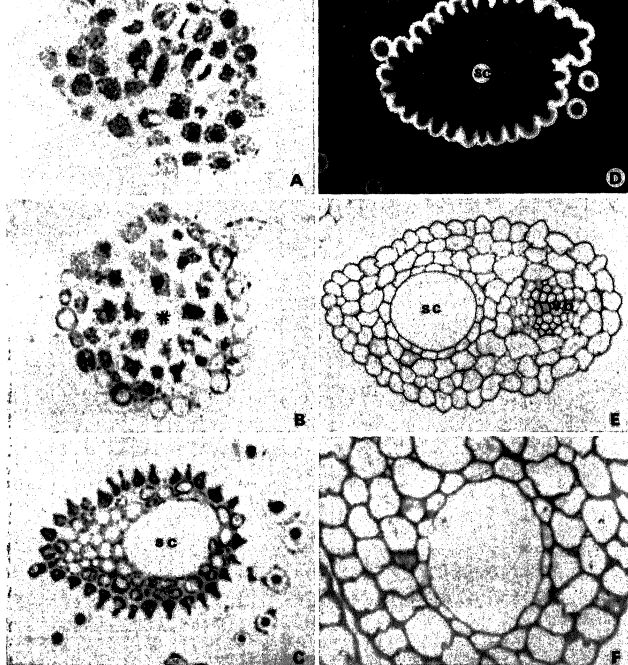


Figure 2. A. TS of the stigma at the base stained with toluidine blue to show the cells of the transmitting tissue with large intercellular spaces formed by the converging stigmatic papillae. B. TS of style just below the stigma. A small cavity has appeared at the centre of the transmitting tissue (asterisk). C. TS style further down the stigma. The stylar canal (sc) has enlarged and is not lined with well-demarcated canal cells. D. TS style a few mm below the stigma stained with auramine O. The stylar canal is not lined with a cuticle; a thick cuticle is obvious on the outer surface of epidermal cells. E. TS middle portion of style stained with PAS reagent. Stylar canal is lined with well-demarcated layer of canal cells. Vb—vascular bundle. F. Similar to E but stained with coomassie blue. Canal cells are non-glandular and resemble cortical cells.

continuous as revealed by PAS staining (figure 2E). It does not show any thickening of the inner tangential wall facing the canal.

The cortical cells of the style are loosely arranged with conspicuous intercellular spaces. The epidermal layer is lined externally with a layer of thick cuticle (figure 2D).

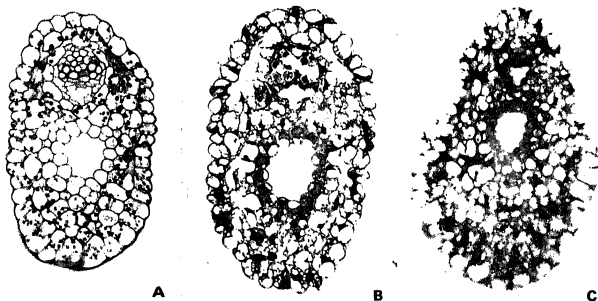


Figure 3. Structure of the pistil in the transitory zone (lower part of the style and upper part of the ovary). **A.** TS lower part of the style stained with PAS. The starch grains are clear in the cortical and epidermal cells. The canal cells are large and are free from starch grains. **B.** As in **A**, but further down, stained with coomassie blue. The canal cells are filled with dense cytoplasm and prominent nuclei. **C.** TS style at the tip of the ovary, stained with coomassie blue. The canal cells are completely filled with dense cytoplasm. A few cells surrounding the layer of canal cells are also prominent.

Non-receptive hairs on the style are also lined with a layer of thick cuticle. The style has a dorsal vascular bundle traversing the whole length which continues into the ovary. The structure of the style is basically similar along almost the whole length.

The lower part of the style enlarges and gradually merges with the ovary. The details of this zone are distinct from the rest of the style. The canal cells in this region are large, glandular with dense cytoplasm and prominent nuclei (figures 3A–C). A few cortical cells surrounding the layer of canal cells also become prominent with dense cytoplasm. As the ovary is approached, the glandular cells increase in size and the canal eventually leads into the ovarian cavity.

Both in the lower part of the style and in the ovary, the cells of the cortex contain abundant starch grains (figure 3A). In the cells of stigma and upper part of the style, however, starch grains are almost completely absent.

Cytochemical techniques do not reveal any contents in the styler cavity. As these studies are confined to the fixed and processed material, the possibility of removal of the styler contents during processing is not ruled out.

4. Discussion

Studies conducted so far on the details of the pistil of many taxa belonging to Papilionoideae of Leguminosae have shown considerable variation in finer details, although they are similar in basic features. The stigma is generally wet and papillate (see Huxley-Harrison and Shivanee, 1977). The exudate may be copious as in *Vicia* and

exudate, the secretion appears on the stigma surface only after the cuticle is disrupted (Heslop-Harrison J and Heslop-Harrison Y 1984). The pistil of *Arachis hypogaea*, although basically resembling that of other Papilionaceous taxa, shows many important variations. Unlike all other taxa so far studied, there is no visible exudate on the stigma of *A. hypogaea*. The papillae are covered by a layer of pellicle similar to those of other taxa with a dry stigma. Also, in contrast to unicellular papillae of other taxa, stigmatic papillae of *A. hypogaea* are multicellular and multiseriate.

The style, although hollow in all Papilionaceous taxa, shows many variations. In *Vigna unguiculata* (Ghosh and Shivanna 1982) the upper part of the style is typically solid with a strand of transmitting tissue. Further down, an irregular cavity appears in the centre of the transmitting tissue by dissolution of the cells. The cavity gradually enlarges towards the lower part of the style and eventually the transmitting tissue becomes confined to 1-3 layers bordering the cavity. This feature is maintained throughout the lower part of the style.

In many other taxa studied (*Trifolium*, Heslop-Harrison Y and Heslop-Harrison J 1982; Heslop-Harrison J and Heslop-Harrison Y 1982; *Cajanus*, Ghosh and Shivanna 1982; *Cicer*, Malti and Shivanna 1983; and *Crotalaria*, Malti and Shivanna 1984), almost the entire style is hollow. The styler canal is bordered by one or a few layers of glandular cells, the canal cells, which are densely cytoplasmic. In *Arachis* also the style is hollow throughout its length. One of the important structural differences of the style in *Arachis*, when compared to other taxa, is the absence of glandular cells bordering the styler canal. The canal cells are vacuolate with scant cytoplasm and resemble cortical cells. Extracellular proteinaceous material on the surface of the inner tangential wall of the canal cells appears to be absent. However, canal cells in the upper part of the ovary become glandular. They are densely cytoplasmic with large nuclei.

Studies conducted so far on the details of the pistil in different groups of plants, have invariably shown the presence of extracellular secretion products in the path of the pollen tubes in the style (either in the styler canal or in the intercellular spaces of the transmitting tissue). Much evidences suggest that these extracellular components, apart from providing nutrition to the growing pollen tubes, are involved in incompatibility responses (see Shivanna 1979, 1982).

In both *Trifolium* (Heslop-Harrison J and Heslop-Harrison Y 1982) and *Crotalaria* (Malti and Shivanna 1984) in which pollen tubes have been traced in the styler canal throughout its length, the styler canal contains extracellular components either in the styler-fluid or as a layer on the inner surface of canal cells. Post-pollination secretion also seems to occur as indicated by the collapse of the surrounding canal cells. Based on fluorescent microscopic studies of wholemounts of pistils (stained with aniline blue), Hawkins and Evans (1973) reported that in *Phaseolus coccineus*, pollen tubes grow in the vicinity of the vascular tissue of the style. They suggested that pollen tubes depend on the phloem for their nutrition. Structural details of the pistil of *Phaseolus* are not available.

As pointed out earlier, in *Arachis*, except in the region just below the stigma and the upper part of the ovary, there is no visible secretion product in the styler cavity. There is not much scope for post-pollination secretion, as the canal is not lined by glandular cells. It is quite possible that the pollen tubes after traversing a short distance in the styler canal just below the stigma, may grow in the vicinity of the vascular bundle as has been reported in *P. coccineus*. This aspect needs to be studied.

- Feder N and O'Brien T P 1968 Plant microtechnique: some principles and new methods; *Am. J. Bot.* **55** 123-142
- Ghosh S and Shivanna K R 1982 Anatomical and cytochemical studies on the stigma and style in some legumes; *Bot. Gaz.* **143** 311-319
- Hawkins G F and Evans A M 1973 Elucidating the behaviour of pollen tubes in intra- and inter-specific pollinations in *Phaseolus vulgaris* L. and *P. coccineus* Lam.; *Euphytica* **22** 378-385
- Heslop-Harrison J and Heslop-Harrison Y 1982 Pollen-stigma interaction in the Leguminosae: The organization of the stigma in *Trifolium pratense* L.; *Ann. Bot.* **51** 571-583
- Heslop-Harrison J and Heslop-Harrison Y 1984 Stigma organization and control of fertilization in *Phaseolus*; in *Proceedings Eucarpia Meeting on Phaseolus Bean Breeding* (ed.) Reimann-Philipp pp 88-96
- Heslop-Harrison J, Knox R B and Heslop-Harrison Y 1974 Pollenwall proteins: exine held fractions associated with the incompatibility response in Cruciferae; *Theor. Appl. Genet.* **44** 133-137
- Heslop-Harrison Y 1977 The pollen-stigma interaction: Pollen tube penetration in *Crocus*; *Ann. Bot.* **41** 913-922
- Heslop-Harrison Y and Heslop-Harrison J 1982 Pollen stigma interaction in the Leguminosae: the secretory system of the style in *Trifolium pratense* L.; *Ann. Bot.* **50** 635-645
- Heslop-Harrison Y and Shivanna K R 1977 The receptive surface of the angiosperm stigma; *Ann. Bot.* **41** 1233-1258
- Jensen W A 1962 *Botanical Histochemistry* (London: W H Freeman and Co)
- Lord E M and Heslop-Harrison Y 1984 Pollen-stigma interaction in the Leguminosae: Stigma organization and breeding system in *Vicia faba* L.; *Ann. Bot.* **54** 827-836
- Lord E M and Webster B D 1979 The stigmatic exudate of *Phaseolus vulgaris* L.; *Bot. Gaz.* **140** 266-271
- Mattsson O, Knox R B, Heslop-Harrison J and Heslop-Harrison Y 1974 Protein pellicle of stigmatic papillae as a probable recognition site in incompatibility reactions; *Nature (London)* **247** 298-300
- McGuckin W F and McKenzie B F 1958 An improved periodic acid Fuchsin sulfite staining method for evaluation of glycoproteins; *Clin. Chem.* **4** 476-483
- Malti and Shivanna K R 1983 Pollen-pistil interaction in chickpea; *Int. Chickpea Newslett.* No. 9 10-11
- Malti and Shivanna K R 1984 Structure and cytochemistry of the pistil of *Crotalaria retusa* L.; *Proc. Indian Nat. Sci. Acad.* **B50** 92-102
- Sastri D C 1984 Incompatibility in angiosperms: Significance in crop improvement; *Adv. Appl. Biol.* **10** 71-111
- Sastri D C and Moss J P 1982 Effect of growth regulators on incompatible crosses in the genus *Arachis* L.; *J. Exp. Bot.* **33** 1293-1301
- Scandalios J G 1969 Genetic control of multiple molecular forms of enzymes in plants: a review; *Biochem. Genet.* **3** 37-79
- Shivanna K R 1979 Recognition and rejection phenomena during pollen-pistil interaction; *Proc. Indian Acad. Sci. (Plant Sci.)* **88** 115-141
- Shivanna K R 1982 Pollen-pistil interaction and control of fertilization; in *Experimental Embryology of Vascular Plants* (ed) B M Johri (Berlin: Springer-Verlag) pp 131-174
- Shivanna K R and Johri B M 1985 *The Angiosperm Pollen: Structure and Function* (New Delhi: Wiley Eastern)
- Weber K and Osborn M 1975 Proteins and sodium dodecyl sulfate: molecular weight determination on polyacrylamide gels and related procedures; in *The Proteins* (eds) H Neurath and R L Hill (New York: Academic Press) **1** 179-223

The Status of *Plagiochasma intermedium* L. et G. in India

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Abstract. *Plagiochasma intermedium* grows commonly in various parts of the country with minor local variations. A critical assessment of the taxonomically significant characteristics show that the plants growing in western Himalaya, central India and peninsular India, with elaters devoid of thickening bands, are all referable to this species. The SEM details of the spore also confirm this conclusion.

Keywords. Bryophyta; Hepaticae; Marchantiales; Rebouliaaceae; *Plagiochasma*.

1. Introduction

The genus *Plagiochasma* is one of the most conspicuous liverworts being luxuriantly distributed in several parts of the country. The various species form two natural groups: (i) those with elaters having spiral thickening bands and (ii) those with elaters devoid of spiral thickening bands.

The latter group sometimes shows irregularly developed faint hyaline bands which never attain the typical pattern of the brown to dark pigmentation of the normal spiral thickening bands. *P. pauriana* and *P. intermedium*, falling in the second category, are known from India (Udar and Chandra 1964, 1965). An examination of recent collections from western ghats in Maharashtra and Kerala, Pachmarhi in central India, Mount Abu in Rajasthan and several localities in western Himalayas have shown that there is a wide distribution of plants with the elaters devoid of spiral thickening bands. The plants from different localities show minor differences in thallus size, epidermal cells, scales, spores and elaters. The plants from Maharashtra are particularly much larger in size, with stalks of archegoniophores rather robust but small in length and with spores smallest in size and lighter in colour, tending to represent a distinctive taxon. However, a critical comparative assessment of specimens from all the collections clearly shows that the various populations occurring in the country, in general, merely reflect local variations and clearly belong to *P. intermedium*.

In a recent contribution, Bischler (1979a) treats *P. pauriana* Udar et Chandra as a synonym of *P. japonicum*. She also regards *P. intermedium* to be common only in America and states that all Asiatic reports by Evans (1915), Kashyap (1929) and Udar and Chandra (1965) do not belong to this species but to *P. japonicum*.

2. Observations

2.1 *Thallus*

The plants from Maharashtra have the largest thalli ranging in length from 8.00–21 mm and in width from 4.00–9.00 mm (figures 1, 2), whereas plants from other localities range in length from 4–18 mm and in width from 2–4.9 mm (figures 19, 22, 23). The stalks of archegoniophores are robust but short in Maharashtra plants (figure 4) and long, usually 5 mm, sometimes more in length also, in plants from other localities. The number of involucres in a disc is minimum (upto 3) in Pachmarhi plants (figures 19–23), and maximum (1–5 in number) in Pauri plants, whereas in plants from other localities it is 2–4 (figures 2–4).

2.2 *Epidermal peeling*

The epidermal cells are polygonal with walls and angles thickened. The plants from Maharashtra and Naini Tal show much thickened walls and well developed trigones (figures 12, 36), whereas in plants from Pachmarhi and Pauri the cells are thin-walled with fine and conspicuous trigones (figure 26).

2.3 *Epidermal pore*

The epidermal pores in plants from all the localities are bounded by 3–4 rings of 7–8 cells each (figures 11, 26, 35) except those from Pauri where the rings of cells may also be reduced to 2 (Udar and Chandra 1964).

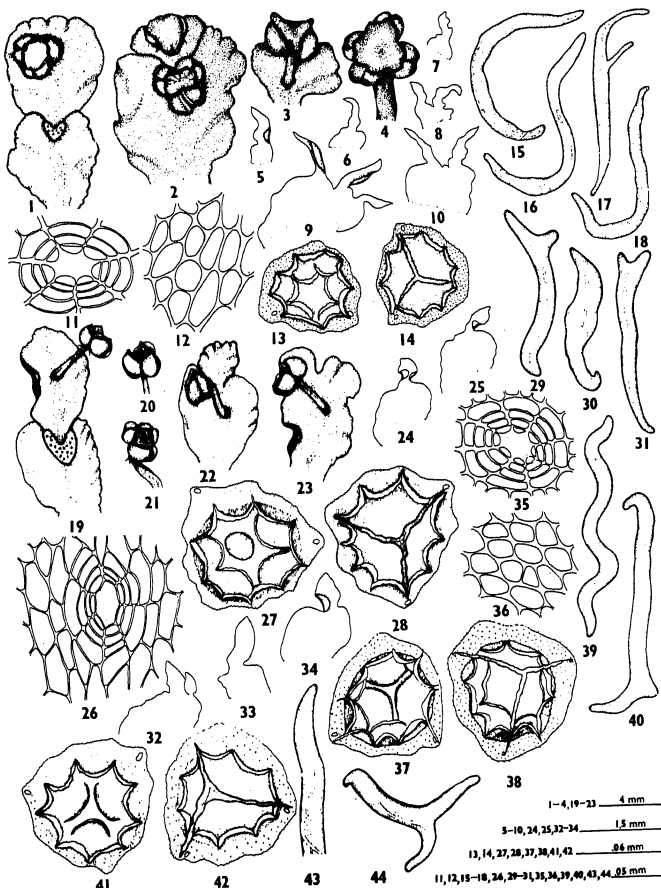
2.4 *Scales*

The scales are purplish with oblong to ovate appendages. The number and shape of appendages vary in plants from different localities. The plants of Maharashtra have been found with 1–3 oblong appendages (figures 5–10). Naini Tal and Pauri plants have scales with 1–2 oblong-ovate appendages (figures 32–34), whereas scales with only one ovate appendage commonly occur in Pachmarhi plants (figures 24, 25).

2.5 *Spores*

The spores are brown, lamellate and with a broad wing. The surface of the wing and the lamellae are either granular or spinous (figures 13, 14, 27, 28, 37, 38, 41, 42). The lamellae form complete to incomplete reticulations on the distal face. The number of alveoli formed is usually 2–3 across the diameter. The proximal face has conspicuous tri-
radiate mark and the lamellae are usually arranged along the periphery.

The plants collected from different localities have spores of the above basic pattern with slight variations in colour, size, margin of the wing and general spore surface. The spores (under LM) are usually brown in colour except the spores of plants from Maharashtra where they are yellow to light-brown. The spores from Pachmarhi and most of same size range (67–90 μm), whereas they are of smaller range in Maharashtra plants and larger in Pauri plants being up to 96 μm . The



Figures 1-44. *P. intermedium*. 1-18. Plants from Maharashtra. 19-31. Plants from

is present in spores of other localities. The spines present in Naini Tal plants are slightly larger in size (figures 37, 38). A triangular area at the summit of tri-radiate mark has also been noted in plants from all localities (figures 28, 42) except those from Naini Tal and Maharashtra (figures 14, 38). The plants collected from Rajasthan and Kerala have spores similar to Pachmarhi plants.

2.6 *Elaters*

The elaters are usually devoid of spiral thickening bands but some elaters in plants from Pauri and Maharashtra (figures 15, 16, 18, 43) have been noted with faint pseudospiral thickening bands, never attaining the brown colour of normal thickening. The large elaters, up to 240 μm long, have been noted in Pachmarhi plants whereas the plants from other localities have elaters only up to 210 μm in length. The elaters in plants from Maharashtra are yellow to light brown in colour whereas they are usually brown in Pachmarhi and Naini Tal plants and comparatively darker in Pauri plants.

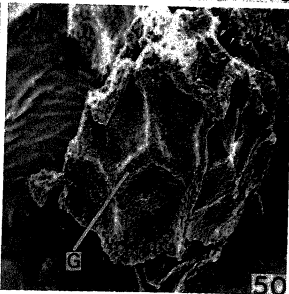
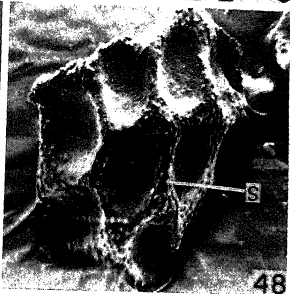
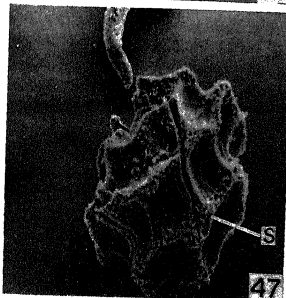
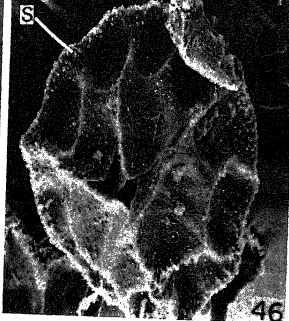
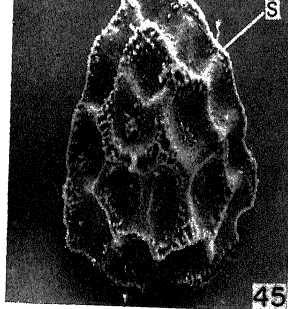
2.7 *SEM details of spores*

The SEM details of spores from Maharashtra, Pauri and Pachmarhi show identical sporoderm architecture. The distal face of spores shows 2-3 alveoli across the diameter. The general lamellar surface is studded with small and blunt spines in Maharashtra and Pauri plants (figures 45-49: S) and granular in Pachmarhi plants (figure 50: G).

2.8 *Specimens examined*

Hepaticae Selectae et Criticae, 517, *Plagiochasma japonicum* St.; Loc.: Japonia, Hondo, Prov. Kotsuke; K. Sakurai fide I Horikawa. 2396, *P. intermedium*, Loc.: Isschoochi Kumamoto Country; Coll. K. Mayebar, July 24, 1950, Det.: K. Mayebar. Annotated as *P. japonicum* by Bischler, 1975. 7913, *P. intermedium*, Loc.: Kiushiu, Kumamoto, Kuma, Koonose; Coll. K. Mayebar; Det.: S. Hattori. Annotated as *P. japonicum* by Bischler, 1975. 495, *P. intermedium*, Loc.: Mt. Saitama; Coll. D. Shimizu, Sept. 2, 1952; Det.: S. Hattori. 8193, 8194, 8195, 8141 *P. intermedium*, all specimens sterile; Loc.: Guatemala IV.

LWU 9124 (Type), 9125 (Isotype), *P. pauriana*; Loc.: Pauri; Coll. R. Udar; Det.: R. Udar and V. Chandra. LWU 5225/81, *P. intermedium*, Loc.: Majkhali (Ranikhet); Coll. S. C. Srivastava, Oct. 8, 1981; Det.: R. Udar and A. Agarwal. LWU 5169/81, *P. intermedium*, Loc.: Panchgani (Maharashtra); Coll. R. Udar and Party, Sept. 26, 1981; Det.: R. Udar and A. Agarwal. LWU 5249/82, *P. intermedium*, Loc.: Pachmarhi; Coll. A. Kumar, D. Kumar and U. S. Awasthi, March 2, 1982; Det.: R. Udar and A. Agarwal. LWU 6353/82, 6364/82, *P. intermedium*, Loc.: Peermade (Kerala); Coll. R. Udar and Party, Sept. 29, 1982; Det.: R. Udar and A. Agarwal. LWU 6793/82, 6794/82, *P. intermedium*, Loc.: Mt. Abu (Rajasthan); Coll. N. K. Mehrotra and Party, Dec. 1982; Det.: R. Udar and A. Agarwal.



Figures 45–50. *P. intermedium*. 45–47. Spores of plants from Pauri. 48, 49. Spores of plants from Maharashtra. 50. Spore of plant from Pachmarhi. 45. Spore in distal face $\times 341$. 46. Spore in proximal face $\times 309$. 47. Spore in distal face with a part of elater $\times 325$. 48. Spore in distal face $\times 616$. 49. Spore in distal face $\times 623$. 50. Spore in proximal face $\times 627$. S, spines; G, granules.

The examination of several collections of *P. intermedium* and *P. japonicum* clearly shows that they distinctly differ in vegetative structures as well as in spore and elater morphology. The basic differences between *P. intermedium* and *P. japonicum* occur in thallus size and number of appendages in ventral scales. In spore morphology, *P. japonicum* has spores with complete reticulations on distal as well as on proximal faces. The walls of reticulations are high with tubercles on the surface, whereas in *P. intermedium* spores have complete as well as incomplete reticulations with relatively low walls. Except vegetative structures and spore morphology both the species show minor differences in elaters also. The elaters in *P. intermedium* are totally devoid of spiral thickening bands (Bischler 1979b) whereas in *P. japonicum* they are very rarely interrupted by spirals (Bischler 1979a). The elater of *P. intermedium* (in Bischler 1979b, figures 13: 8) however, shows conspicuous spiral thickening which may probably belong to *P. japonicum*.

The Indian plants do resemble *P. japonicum* but in spore morphology, they are closely related to *P. intermedium* and distinctly differ from *P. japonicum*. It is also true that plants of *P. pauriana* do not resemble with *P. intermedium* in totality in vegetative structure and spore size but considering the range of variations it is similar to the latter and not to *P. japonicum*.

The SEM details of spores of various Indian populations (figures 45–50) and of *P. japonicum* (Bischler 1979a, figures 6: 4–6) show different sporoderm ornamentation. The sporoderm of the Indian population shows close relationship with Mexican *P. intermedium* in having relatively larger and lesser number of complete-incomplete reticulations with low walls across the diameter of the spore (see Bischler 1979b, figures 13: 1, 3). The spores of *P. japonicum*, however, have smaller alveoli which are also more in number across the spore diameter. The lamellae forming reticulations are relatively high and broader having closely studded papillae giving a some what pseudoreticulate appearance. Some of the Japanese specimens examined by us also showed close relationship with *P. intermedium* in sporoderm morphology. Some of the SEM details of spores given for Japanese *P. japonicum* (in Bischler 1979a, figures 6: 1, 2) resemble *P. intermedium*. Therefore, *P. japonicum* appears to be a genuine species and both *P. intermedium* as well as *P. japonicum* occur in Japan but the latter does not seem to occur in India as it has not yet been collected from this territory, and the Indian plants undoubtedly belong to *P. intermedium*.

Acknowledgements

The authors are indebted to late Professor Ram Udar for critically evaluating the identity of *P. intermedium* and *P. japonicum* without which the present work would have not been completed. Grateful thanks are also due to Naturhistorisches Museum, Botanische Abteilung, Berging, WIEN for the loan of specimens of *P. intermedium*, to Mr V K Lal, IIT, Delhi for the SEM details, to the University Grants Commission and to the Department of Science and Technology, New Delhi for financial assistance.

References

- Bischler H 1979a *Plagiochasma* L. et L. III Les taxa D'Asie et D'oceanie; *J. Hattori Bot. Lab.* **45** 25–79
Bischler H 1979b *Plagiochasma* L. et L. IV Les taxa americains; *Rev. Bryol. Lichenol.* **45** 255–333

- Evans A W 1915 The genus *Plagiochasma* and its North American species; *Bull. Torr. Bot. Club* **42** 259–306
- Kashyap S R 1929 *Liverworts of the western Himalayas and the Panjab plains I*; Lahore
- Udar R and Chandra V 1964 A new species of *Plagiochasma*, *P. pauriana* Udar et Chandra, from Pauri Garhwal, India; *Rev. Bryol. Lichenol.* **33** 213–215
- Udar R and Chandra V 1965 Morphology and life history of *Plagiochasma intermedium* L. et G.; *J. Hattori Bot. Lab.* **28** 75–93

Cytological studies in *Sonchus oleraceus* Linn.

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Abstract. *Sonchus oleraceus* Linn. The cytological study of a few of the collections of *Sonchus oleraceus* has been carried out. All the collections of *Sonchus oleraceus* showed $2n = 32$ chromosomes. 'Chromosome mosaics' is noticed in one of the root tip studies. There is a wide range in variation with regard to the position of centromeres in the chromosomes of the somatic complement. Sixteen bivalents are counted at diakinesis. Occurrence of 'chromosome mosaics' has been discussed.

Keywords. *Sonchus oleraceus*; cytology; Compositae.

1. Introduction

Sonchus oleraceus Linn. is the type genus (Boulos 1960) of Compositae. The plant is an erect robust milky herb growing to a height of 2–3 ft or more and bears thistle-shaped heads with yellow flowers. A number of workers have made cytotaxonomical studies in the genus *Sonchus* (Boulos 1960; Chatterjee and Sharma 1969; Tsun-shih Hsieh *et al* 1972). However, cytological studies with respect to geographical distribution of *S. oleraceus* has not been carried out. An attempt is made in this direction.

2. Materials and methods

The plants were collected for the present work from different places during our study tours. Root-tips were treated with 0.002 M. 8-Hydroxyquinoline, squashed with aceto-orcein as per the method given by Tjio and Levan (1950). Flower buds of suitable size were fixed in acetic alcohol (1:3). The anthers were squashed in a drop of 1% acetocarmine. The slides were made permanent by using butyl-alcoholic acetic series (Celarier 1956). To determine the centromere position the terminology given by Levan *et al* (1964) was adopted.

3. Observations

The Karyotype in each of the collections consists of $2n = 32$ chromosomes, except in Coll. No. 107 (Banasankari, about 100 km) the karyotype shows $2n = 32$ and 64 chromosomes and 16 bivalents at diakinesis (figures 1–11). Further observations are given here:

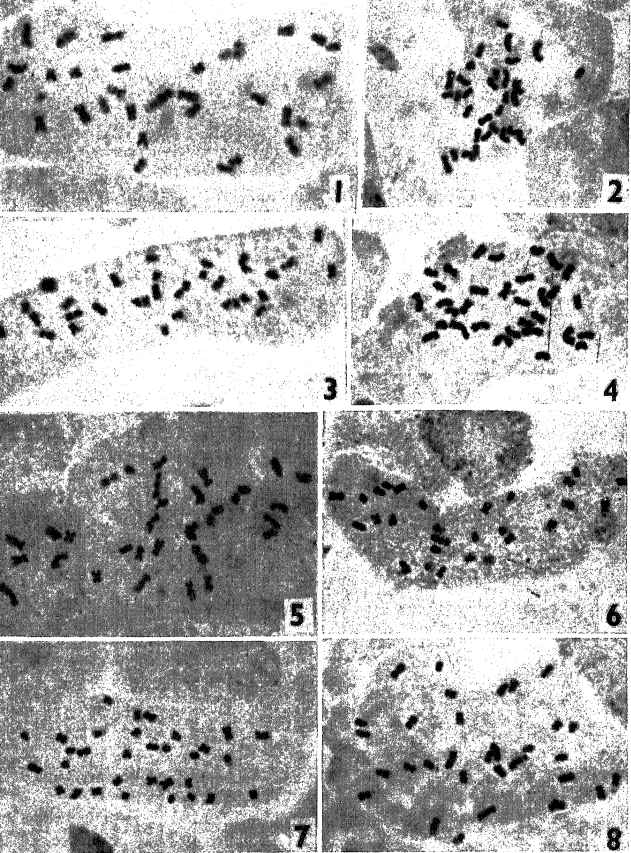
Coll. No. and location	Median stricto centromere	Median region centromere	Sub-median centromere	Range in length of chromosomes	Absolute length of chromosomes	Satellites and their position on arms of chromosomes
127 Dharwad	—	13 pairs	3 pairs	3.11–1.28 μ	69.32 μ	Two pairs of satellites One pair on short arm, another on long arm
145 Sudi	One pair	15 pairs	—	3.51–1.28 μ	68.30 μ	Nil
90 Gopankopp	—	11 pairs	5 pairs	2.29–1.15 μ	72.14 μ	Two pairs SATS, both on short arms
126 Saundatti	—	16 pairs	—	2.77–1.22 μ	62.16 μ	Nil
163 Kesarbhavi	Two pairs	11 pairs	3 pairs	3.31–1.08 μ	71.46 μ	Nil
102 Dandeli	One pair	12 pairs	3 pairs	2.16–1.08 μ	49.00 μ	Two pairs of SATS on short arms
73 Kemangundi	One pair	12 pairs	3 pairs	2.16–1.08 μ	49.00 μ	Two pairs of SATS on short arms
69 Kemangundi	—	11 pairs	5 pairs	2.77–1.35 μ	65.06 μ	Two pairs of SATS on short arms

4. Discussion

Taxonomists have been giving importance to the studies in chromosome numbers and their variations. Polyploidy is the commonest variation in vascular plants and Stebbins (1956) has estimated the percentage of polyploidy in angiosperms in general as 30–35%. However, polyploidy is considerably less in Compositae according to Morton (1966).

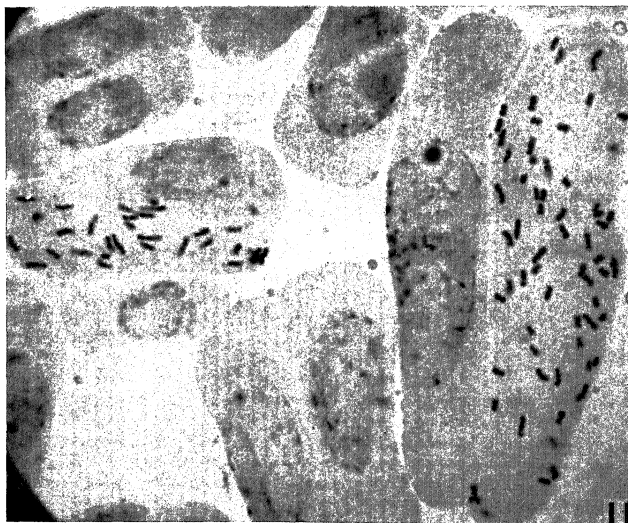
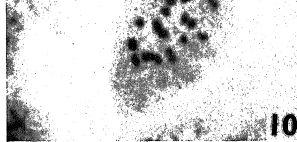
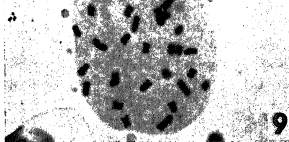
In the collections of *Sonchus oleraceus*, the somatic chromosome number is found to be $2n = 32$. However, in collection No. 107 of *S. oleraceus* $2n = 32$ and $2n = 64$ chromosomes were seen from the cells of the same root tip (figure 11). There is also a variation in the size of the cells and the nuclei in them. The cells containing $2n = 64$ chromosomes are approximately twice as large as the cells containing 32 chromosomes. The nucleus in such cells is also approximately twice as large as those of normal cells. This variation, perhaps, is due to endoduplication. Variation of chromosome number within the same tissue is termed as 'chromosome mosaics' (Frankhauser 1945). 'Chromosome mosaics' are reported in the hybrids in Graminae as well as in other families. Different chromosome numbers in pollen mother cells have been recorded in polyploids and experimental amphidiploids of *Triticum*, *Aegilops* and *Agropyron* by Sachs (1952), in *Secale* by Rees and Thompson (1955). Chopra (1960) reported that in one monosomic line *Triticum aestivum* different PMC's had numbers ranging from 6–21.

Shahre and Shastry (1963) also recorded such mosaics in tetraploid garden roses. Malik and Thomas (1966) recorded chromosome mosaic in *Narenga porphyracoma*.



Figures 1-8. Photomicrographs of Coll. Nos. 127, 145, 90, 126, 163, 102, 73 and 69 respectively all showing $2n = 32$. All Mitosis $\times 1200$.

Nair (1972) observed chromosome mosaics in the pollen mother cells of *Saccharum officinarum* \times *S. montanum* hybrids. Gupta and Srivastava (1972) reported chromosome mosaics in one of collections of *Paspalum paspaloides*. Reports of chromosome mosaics



Figures 9–11. 9. Photomicrograph of somatic metaphase plate of Coll. No. 107 ($2n = 32$). 10. Diakinesis showing 16_{II} Coll. No. 107. 11. Photomicrograph of somatic metaphase plate of Coll. No. 107 showing $2n = 32$ and $2n = 64$ in different cells of the same root-tip. Mitosis $\times 1200$. Meiosis $\times 920$.

Yung 1964), and in *Saccharum officinarum* (Jagathesan and Ratnambal 1967) are on record.

Possibly Coll. No. 107 *S. oleraceus* is a hybrid origin.

According to Darlington and Wylie (1955) and Tomb (1977) the genus is tribasic with basic numbers as $X = 7, 8$ and 9 .

S. oleraceus with $2n = 32$ chromosomes is probably a tetraploid taxon with basic chromosome number $X = 8$. The work of Tsun-Shih Hsieh *et al* (1972) indicated that *S. oleraceus* is an autotetraploid rather than allotetraploid as reported by Stebbins *et al* (1953). The present work supports the former view.

In the somatic complement of chromosomes, chromosomes with median region

these are found to vary in their number in different populations. There is a slow change in the position of the centromeres. This is due, probably, to repatterning of the chromosomes which is taking place in somatic complement. It leads to marked changes in karyotype (Huziwar 1959; Stebbins *et al* 1953).

In some collections satellites are not seen. This process of loss has occurred in many species where the tetraploids have a single pair (Gates 1942). Sometimes, it is possible SAT can also be lost by gradual diminution until it is below the limits of visibility. Further, satellited chromosomes are not the longest in the somatic complement. This is in accordance with the observations of Anderson (1966) in *Chrysanthamnus*. Of the two pairs of SATs in Coll. No. 127, one pair was on long arm and another pair was on short arm. This unusual position of SATs on long arms is possibly due to translocation (Jackson 1971).

References

- Anderson L C 1966 Cytotaxonomic studies on *Chrysanthamnus* (Asteraceae, Compositae); *Am. J. Bot.* **53** 204-212
- Boulos L 1960 Cytotaxonomic studies in the genus *Sonchus* 2. The genus *Sonchus*, a general systematic treatment; *Bot. Not.* **113** 400-420
- Britton D M and Hull J W 1957 Meiotic instability in *Rubus*; *J. Hered.* **48** 11-20
- Celariar R P 1956 Tertiary butyl alcohol dehydration of chromosome smears; *Stain Technol.* **31** 155-157
- Chatterjee T and Sharma A K 1969 Cytotaxonomy of Cichorieae; *Genetika* **40** 577-590
- Chopra V L 1960 Premeiotic somatic reduction in wheat; *Curr. Sci.* **29** 362-364
- Darlington C D and Wylie A P 1955 *Chromosome Atlas of Flowering Plants* (George Allen and Unwin Ltd)
- Frankhauser G 1945 The effect of changes in chromosome numbers on amphibian development; *Q. Rev. Biol.* **2** 20-78
- Gates R R 1942 Nucleoli and related nuclear structure; *Bot. Rev.* **8** 337-409
- Gupta P K and Srivastava A K 1972 Aberrant meiosis and spindle abnormalities in *Paspalum paspaloides* (Michx.); *Serin. Genetika* **43** 76-83
- Huziwar Y 1959 Chromosomal Evolution in the subtribe Asterinae; *Evolution* **13** 188-193
- Jackson R C 1971 The Karyotype in systematics; *Annu. Rev. Ecol. Syst.* **2** 327-368
- Jagathesan D and Rathanam M J 1967 Karyotype analysis in *Saccharum officinarum*; *the Nucleus (Calcutta)* **10** 159-167
- Levan Albert Fredge and Sandberg Avery A 1964 Nomenclature for centromeric position on chromosomes; *Hereditas* **52** 201-220
- Malik C P and Thomas P T 1966 Karyotype studies in some *Lolium* and *Festuca* species; *Caryologia* **9** 167-196
- Morton J K 1966 The Role of Polyploidy in the evolution of Tropical Flora; in *Chromosomes today* (eds) C D Darlington and K R Lewis (Edinburgh, London: Oliver and Boyd) 73-76
- Nair M K 1972 Cytogenetics of *Saccharum officinarum* L. and *S. spontaneum* L. and *S. officinarum* × *S. spontaneum* Hybrids I Chromosome mosaics; *Cytologia* **37** 565-573
- Rees H and Thompson B 1955 Localization of chromosome breakage at meiosis; *Heredity* **9** 359-407
- Sachs L 1952 Chromosome mosaics in experimental amphidiploids in Triticinae; *Heredity* **6** 157-170
- Shahre M L and Shastry S V S 1963 Meiosis in garden roses; *Chromosoma* **13** 702-724
- Shung-Jun-Yung 1964 Numerical chromosomal instability in *Nicotiana* hybrids I. Inter plant variation among offspring of amphidiploids; *Genetics* **50** 745-756
- Stebbins G L 1956 Cytogenetics and Evolution of the grass family; *Am. J. Bot.* **43** 890-905
- Stebbins G L, Jenkins J A and Walters J L 1953 Chromosomes and phylogeny in Compositae; Tribe Cichorieae; *Univ. Calif. Publ. Bot.* **26** 401-430
- Tsun-Shih Hsieh A B, Schooler Allyn Bell and John D Nalewaja 1972 Cytotaxonomy of three *Sonchus* species; *Am. J. Bot.* **56** 789-796
- Tjio J H and Levan A 1950 The use of oxyquinoline in chromosome analysis; *Ann. Estac. Exp. Aula. Dei.* **2** 21-64
- Tomb A S 1977 Lactuceae—systematic review in (ed) V H Heyhood, J B Harborne and B L Turner "The

Gametophytes, integuments initiation and embryogeny in *Microstylis cylindrostachya* (Orchidaceae, Epidendreae)

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Abstract. The development of gametophytes, initiation of integuments and embryogeny are described in *Microstylis cylindrostachya*. The anther wall consists of an epidermis, fibrous endothecium, one middle layer and secretory tapetum with uninucleate cells. Its development corresponds to the Monocotyledonous type. Cytokinesis is simultaneous. The microspore tetrads are decussate, isobilateral and tetrahedral. At shedding, the pollinia are 2-celled. The ovules are anatropous, bitegmic and tenuinucellate. Both the integuments are dermal in origin. Development of the female gametophyte is of the Monosporic type. Double fertilization occurs. The primary endosperm nucleus degenerates. Development of the embryo corresponds to the Asterad type. The mature embryo is undifferentiated. The seed is non-endospermic and the seed coat is formed entirely by the outer layer of the outer integument.

Keywords. *Microstylis cylindrostachya*; gametophytes; integuments initiation; embryo; pericarp; Orchidaceae.

1. Introduction

The genus *Microstylis* (Nutt.) Eaton, subtribe Liparidinae, tribe Epidendreae, subfamily Orchidoideae (Dressler and Dodson 1960), comprises 300 species distributed both in temperate and tropical regions of the world (Willis 1973). This genus is represented in India by 22 species (Hooker 1894). *Microstylis cylindrostachya* Reichb. f. occurs in temperate and subtropical Himalayas.

The embryology of Orchidaceae has attracted the attention of several workers from time to time because of the interesting diversity in the development and organisation of the female gametophyte, characteristics of suspensor, apomixis and polyembryony. The publications of Schnarf (1931), Swamy (1949a, b), Wirth and Withner (1959), Davis (1966), Abe (1972) and Veyret (1974) summarized the previous embryological work on the family. However, a perusal of these works revealed that the genus *Microstylis* is embryologically unknown and hence the present investigation was undertaken.

2. Material and methods

M. cylindrostachya was collected from Fagoo, Mahasu and Shimla during August–September, 1982. Formalin-acetic-alcohol was used as fixative and subsequently the material was stored in 70% ethyl alcohol. Conventional methods of microtechnique were followed. The serial sections were cut at 7–10 μ m and stained with

3. Observations

3.1 *Microsporangium, microsporogenesis and male gametophyte*

The anther is tetralocular (figure 1). The microspore mother cells in a young anther lobe are surrounded by an epidermis and two secondary parietal layers (figure 2). The outer secondary parietal layer (osp) forms directly the endothecium whereas the inner secondary parietal layer (isp) undergoes a periclinal division to form a middle layer and tapetum (figure 2). The anther wall development conforms to the Monocotyledonous type.

Fully differentiated anther wall consists of an epidermis, endothecium, one middle layer and tapetum (figure 3). The tapetum is of the glandular type and its cells are uninucleate (figures 3, 5).

The mature anther wall comprises of an epidermis and endothecium with fibrous thickenings while the middle layer and tapetum degenerate (figure 4).

Cytokinesis in microspore mother cells is of the simultaneous type (figures 6-9) resulting in the formation of isobilateral, decussate and tetrahedral tetrads (figures 3, 10-12). The tetrads remain together to form pollinia. At anthesis, the pollinia are 2-celled (figure 4).

3.2 *Megasporangium*

Numerous ovular primordia develop from the parietal placenta (figures 13, 36) and each consists of an axial row of six to eight cells surrounded by an epidermis (figure 13).

At the archesporial cell stage the inner integument initials arise from the ovular epidermis (figure 14). The initials consist of two adjacent dermal cells which by periclinal and anticlinal divisions, give rise to two cells thick inner integument (figures 15, 16). The micropyle is formed by the inner integument (figure 24).

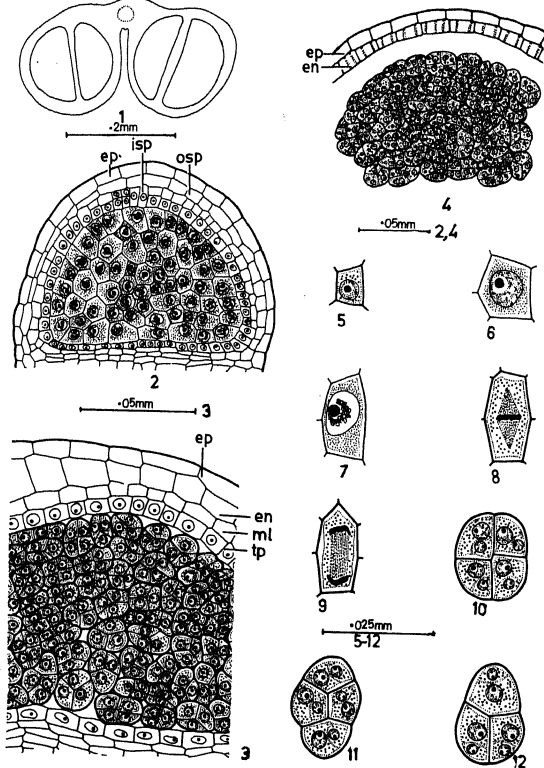
The outer integument primordium differentiates close to the base of the inner integument, and is formed exactly like the inner one (figures 15, 16). In the prefertilization stages the outer integument is behind the inner one (figure 24). However, it grows beyond the inner one in the postfertilization stages. The mature ovules are anatropous, bitegmic and tenuinucellate (figure 24).

3.3 *Megasporogenesis and female gametophyte*

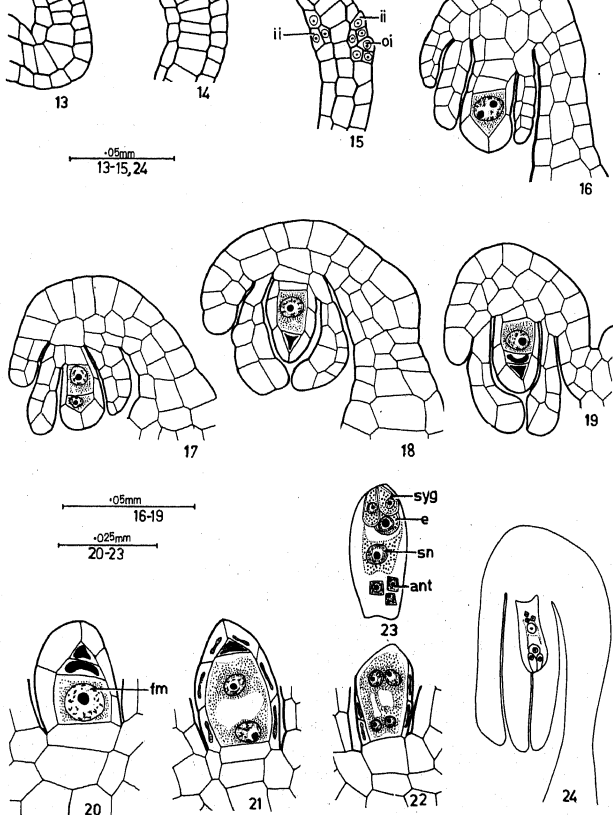
The hypodermal archesporial cell functions directly as the megaspore mother cell (figures 13, 16). It undergoes the first meiotic division to form a dyad with a small micropylar and a large chalazal cell (figure 17). The micropylar cell of the dyad degenerates (figure 18). The chalazal dyad cell undergoes the second meiotic division resulting in two megaspores (figures 19, 20). The chalazal, functional megaspore undergoes 3 successive mitotic divisions to form 8-nucleate embryo sac (figures 21-23). The development of embryo sac conforms to the monosporic, Polygonum type.

The mature embryo sac contains an egg apparatus, secondary nucleus and 3 antipodal cells (figure 23).

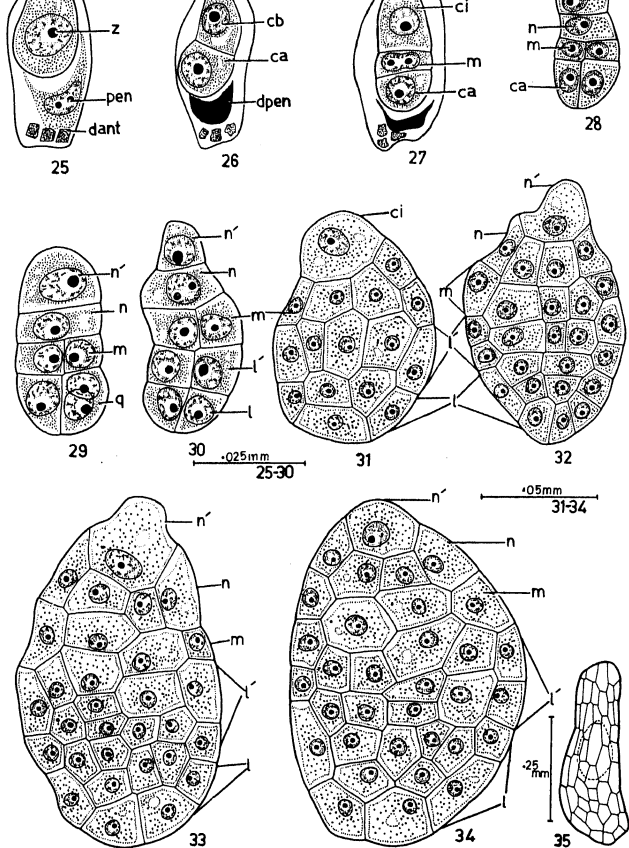
3.4 *Fertilization and endosperm*



Figures 1-12. Microsporangium, microsporogenesis and male gametophyte. 1. Outline diagram of transection of anther. 2. Portion of transection of anther at microspore mother cell stage showing wall layers. 3. Portion of transection of anther at pollen tetrad stage showing wall layers. 4. Portion of transection of mature anther showing epidermis, fibrous endothecium and pollen tetrads. 5. Tapetal cell. 6-9. Meiotic divisions in microspore mother cells. 10-12. Isobilateral, decussate and tetrahedral tetrads. (*en*, endothecium; *ep*, epidermis; *isp*, inner secondary parietal layer; *ml*, middle layer; *osp*, outer secondary parietal layer; *tp*, tapetum).



Figures 13-24. Megasporangium, megasporogenesis and female gametophyte. 13. Ovular primordium containing archesporium. 14, 15. Initiation of integuments in young ovules. 16. Megaspore mother cell. 17. Dyad with small micropylar cell. 18. Dyad with degenerated micropylar cell. 19, 20. Triad showing micropylar two degenerated cells and functional megaspore. 21, 22. Two- and 4-nucleate embryo sacs. 23. Mature embryo sac. 24. Longisection of mature ovule which is anatropous, bitegmic and tenuinucellate. (*ant*, antipodals; *e*, egg; *fm*, functional megaspore; *ii*, inner integument; *oi*, outer integument; *sn*, secondary nucleus; *syg*, synergid).



Figures 25-35. Endosperm and embryogeny. **25.** Zygote, primary endosperm nucleus and degenerated antipodals. **26, 27.** Two- and 3-celled proembryos; note the degenerated primary endosperm nucleus and antipodals. **28-30.** Six-, 8- and 12-celled proembryos. **31, 32.** Stages leading to the formation of globular proembryo. **33, 34.** Young and mature embryos. **35.** Mature seed in surface view. (*dant*, degenerated antipodals; *dpen*, degenerated primary endosperm nucleus; *pen*, primary endosperm nucleus; *z*, zygote).

division occurs in *ca* resulting in a T-shaped proembryo (figure 28). The *ci* divides transversely to form *n* and *n'* (figure 28).

The two daughter cells of *ca* undergo a longitudinal division, at right angles to the first, forming the quadrant *q* (figure 29). The *m* gives rise to two juxtaposed cells after a longitudinal division (figure 28). The cell *n* divides vertically (figure 32). The tier *q* divides to form an octant with two tiers *l* and *l'* (figure 30). Subsequent divisions occur in the embryonic tiers *l*, *l'* and *m* (figures 31–34). Both the suspensor cells *n* and *n'* also take part in the organization of mature embryo. Thus, the mature embryo is formed from the derivatives of *l*, *l'*, *m*, *n* and *n'* (figure 34).

3.6 Seed and seed coat

The seeds are minute, very light and non-endospermic. Each seed contains an embryo surrounded by a thin seed coat (figure 35).

During postfertilization stages the tegmen and the inner layer of testa degenerate. The cells of the outer layer of testa become transparent and form a 1-layered seed coat consisting of vertically elongated, thin-walled cells (figure 35).

3.7 Pericarp

In transection, the ovary (figure 39) shows 3 sterile and 3 fertile valves (figure 36). Each valve possesses a vascular bundle.

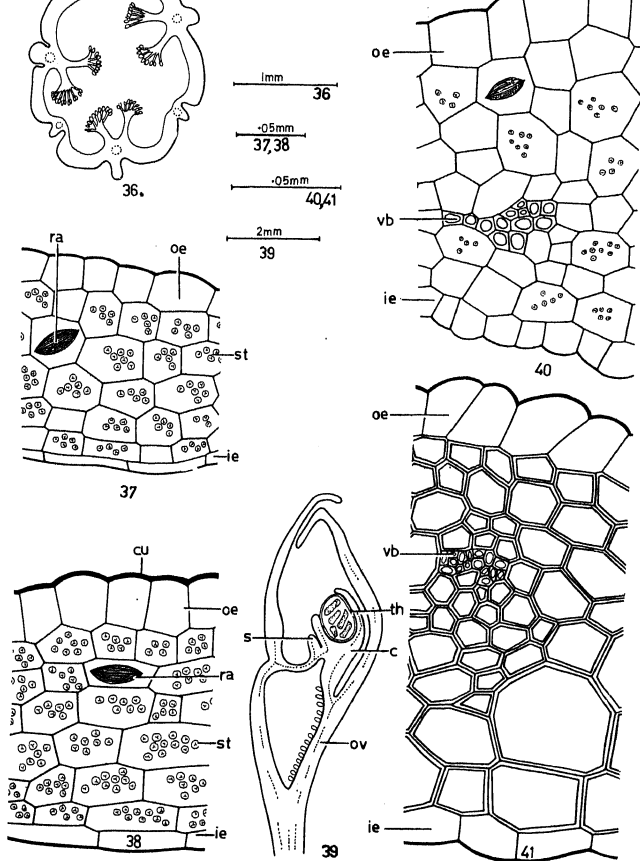
Prior to fertilization, the fertile valve is 6 or 7 layered (figure 37). The cells are thin-walled and contain starch and raphides. In postfertilization stages, the outer wall of the outer epidermis becomes thickened whereas the ground tissue remains parenchymatous (figure 38).

The sterile valve of young ovary consists of 11 layers of parenchyma (figure 40). Some of the cells contain starch and raphides as in fertile valves. In the postfertilization stages the thickness of the valve increases up to 18 cells. The outer epidermis becomes strongly thickened and the whole pericarp becomes sclerenchymatous (figure 41).

4. Discussion

In *M. cylindrostachya*, unlike most orchids (Wirth and Withner 1959), the anther wall is 4-layered thick and shows the Monocotyledonous development of Davis (1966). The present finding is the first record for the tribe Epidendreae. Swamy (1949a) reported the disorganisation of epidermis in the mature anther of orchids. On the contrary, it persists in *Microstylis cylindrostachya* as in *Arundina graminifolia*, *Habenaria densa*, *Neottia listeroides*, *Satyrium nepalense* and *Spathoglottis plicata* (Mohana Rao and Sood 1979a, b; Prakash and Lee-Lee 1973; Rao 1967; Sood 1984). The tapetal cells remain uninucleate as in *Habenaria densa*, *Neottia listeroides* and *Satyrium nepalense* (Mohana Rao and Sood 1979a, b; Sood 1984).

The embryo sac in *M. cylindrostachya* is of the monosporic, 8-nucleate type as in most of the other species of tribe Epidendreae hitherto investigated (Abe 1972; Mohana



Figures 36-41. Pericarp. 36. Transection of ovary showing sterile and fertile valves. 37. Transection of young ovary wall passing through the fertile valve. 38. Transection of pericarp of mature fruit in the region of fertile valve. 39. Median longitudinal section of flower (semi-diagrammatic). 40. Transection of young ovary wall in the region of sterile valve. 41. Transection of pericarp of mature fruit in the region of sterile valve. (c, column; cu, cuticle; ie, inner epidermis; oe, outer epidermis; ov, ovary; ra, raphides; s, stigma; st, starch; th, theca; vb, vascular bundle).

antipodals in *Cypripedium cordigerum* after fertilization in *M. cylindrostachya*. Like most other orchids, the cells of nucellar epidermis and inner integument do not show any enlargement and their cells degenerate in the developing seeds.

According to Swamy (1949b), in orchids only the first two or three cell generations in the zygote are consistent and the subsequent cell divisions are irregular. However *M. cylindrostachya* shows regular cell divisions even after the third cell generation. The mature embryo is organised from the derivatives of terminal cell, middle cell and suspensor initial cell. Thus, its embryogeny corresponds to the Group 'A' (Swamy 1949b) or Asterad type (Johansen 1950). The mature embryo of *A. graminifolia*, *Bletia hyacinthiana*, *B. striata*, *Epidendrum nitellinum*, *Platyclinis glumaceae*, *Polystachya microbambusa* and *Sobralia macrantha* (Rao 1967; Tohda 1968; Treub 1879; Veyret 1965, 1974) shows differentiation of identifiable histogens. In *M. cylindrostachya*, the mature embryo is undifferentiated.

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References

- Abe K 1972 Contributions to the embryology of the family Orchidaceae. VI. Development of the embryo in 15 species of Orchids; *Sci. Rep. Tôhoku Univ.* **36** 135-178
- Davis G L 1966 *Systematic embryology of the angiosperms* (New York: John Wiley)
- Dressler R L and Dodson C H 1960 Classification and phylogeny in the Orchidaceae; *Ann. Mo. Bot. Gdn.* **48** 58-66
- Hooker J D 1894 *The flora of British India* (Kent: L. Reeve and Co. Ltd.) Vol 6
- Johansen D A 1950 *Plant embryology* (Waltham, Massachusetts: Chronica Botanica)
- Mohana Rao P R and Sood S K 1979a Embryology of *Habenaria densa* (Orchidaceae); *Bot. Not.* **132** 145-148
- Mohana Rao P R and Sood S K 1979b Life history of *Satyrium nepalense* (Orchidaceae); *Norw. J. Bot.* **26** 285-294
- Mohana Rao P R and Rao K M 1983 Embryology of *Liparis viridiflora*; *Acta Bot. Ind.* **11** 228-234
- Poddubnaya-Arnoldi V A 1960 Study on fertilization in the living material of some angiosperms; *Phytomorphology* **10** 185-198
- Poddubnaya-Arnoldi V A 1964 Comparative embryological study of members of the family Orchidaceae; *Byull. Gl. Bot. Sada* **54** 51-62
- Prakash N and Lee-Lee Aow 1973 Life history of a common Malaysian orchid *Spathoglottis plicata*; *Phytomorphology* **23** 9-17
- Rao A N 1967 Flower and seed development in *Arundina graminifolia*; *Phytomorphology* **17** 291-300
- Schnarf K 1931 *Vergleichende embryologie der angiospermen* (Berlin: Gebrüder Borntraeger)
- Sood S 1984 An embryological study of *Neottia listeroides*, a saprophytic orchid in India; *J. Plant Anatomy* **1** 69-75
- Swamy B G L 1949a Embryological studies in the Orchidaceae. 1. Gametophytes; *Am. Midl. Nat.* **41** 184-200
- Swamy B G L 1949b Embryological studies in the Orchidaceae. 2. Embryogeny; *Am. Midl. Nat.* **41** 202-231
- Tohda H 1968 Development of the embryo of *Bletia striata*; *Sci. Rep. Tôhoku Univ.* **34** 125-131

Treub M 1879 Notes sur l'embryogénie de quelques Orchidées; *Natuurk. Ver. Der Koninkl. Akadem. Deel*; 19 1-50

Veyret Y 1965 *Embryogenie comparee et blastogenie chez les Orchidaceae-Monandreae* (Paris: Orstom)

Veyret Y 1974 Development of the embryo and the young seedling stages of Orchids: in *The Orchids—scientific studies* (ed.) C L Withner (New York: John Wiley) pp 223-265

Willis J C 1973 *A dictionary of the flowering plants and ferns*; revised by H K Airy Shaw (Cambridge: Cambridge Univ. Press)

Wirth M and Withner C L 1959 Embryology and development in the Orchidaceae; in *The Orchids—a scientific survey*, (ed.) C L Withner (New York: Ronald Press) pp 155-188

Influence of clipping and water stress on growth performance and nutrient value of four range grasses

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Abstract. The paper examines the effect of water stress and clipping treatments on growth behaviour and nutrient value of 4 grasses, viz., *Lolium perenne*, *Poa pratensis* (both C_3 plants), *Chloris gayana* and *Panicum coloratum* (both C_4 plants).

Biomass, net production, relative growth rates were affected more markedly and adversely in the two C_4 species due to water stress. The effect of clipping varied with species and was generally more marked and adverse in two C_4 species. The C_3 plants developed higher R:S ratio under water stress. Water stress resulted in a greater decline of total non-structural carbohydrate and protein content in the two C_4 species. Clipping affected adversely the non-structural carbohydrate content and again the effect was more marked in the two C_4 species. On the other hand, protein content in shoots of all plants increased due to clipping.

Keywords. Clipping; water stress; growth performance; total non-structural carbohydrate; protein content.

1. Introduction

Under conditions of water stress growth of plants is usually reduced (Bassiri *et al* 1977; Yegappan *et al* 1982). The decrease is evidently due to decline in net assimilation brought about by the decreased water potential in the leaves (De Puit and Caldwell 1975). The effect of water stress varies with species (Brown and Blaser 1965). Further C_3 and C_4 plants respond differentially to water stress.

Defoliation is another factor affecting the persistancy of grass plants. Yield, vigour and total non-structural carbohydrate (TNC) levels of plants are drastically reduced by intensive defoliations (Trlica and Singh 1979).

In the present investigation the following 4 grass species were tested for their comparative resistance/susceptibility to water stress and defoliation (clipping treatments): *Lolium perenne* L. and *Poa pratensis* L. (C_3 plants) and *Chloris gayana* Kunth and *Panicum coloratum* L. (C_4 plants). The former two species occur naturally in this area while the latter two are being tested for introduction.

2. Methods of study

Uniform sized tillers of *L. perenne* and *P. pratensis* were collected in July, 1977 from a native sward, while those of *C. gayana* and *P. coloratum* were obtained from the experimental farm of Indo-German Agricultural Development Association (IGADA) where they had been grown for about 10 years. The tillers were transplanted into polyethylene pots filled with a 3:1 mixture of soil and farmyard manure and grown in a glass house at Naini Tal from July-November.

The water holding capacity of the pot mixture was determined (Piper 1966) before the start of the experiment, and after tiller transplantation. For the first two weeks the pots were watered regularly to maintain the soil water at the level of maximum field capacity. After this period soil water content in one set of pots for each species was maintained at field capacity (1FC) while in the other set the soil was allowed to dry to a level as close as possible to half field capacity ($\frac{1}{2}$ FC). Under both conditions pots were weighed every third or fourth day and soil water was brought to the desired level (i.e. 1FC and $\frac{1}{2}$ FC) by adding the required amount of water. In addition to the regular weighing, soil water content was monitored gravimetrically at frequent intervals. For each species 72 pots (one plant per pot) were maintained under 1 FC and 72 under $\frac{1}{2}$ FC. The variation in gravimetric water content within treatments was small (Pande and Singh 1981).

Thereafter, under each water condition, plants of each species were divided into 4 sets (18 pots per set). One set of 18 pots in each case was treated as control (unclipped). Out of the remaining 3 sets, one set each was subjected to weekly, fortnightly and monthly clipping treatments. First clipping was identified as time zero. The height of clipping varied from species to species and was fixed so as to remove 80% shoot, by volume, from each plant on the basis of predetermined height-volume relationships. The clipping height from the base of the tiller was: *L. perenne* 6.6 cm, *P. pratensis* 7.0 cm, *C. gayana* 7.0 cm and *P. coloratum* 9.6 cm. The clipped material was oven-dried and weighed on each treatment date.

Three plants from each treatment were selected at random for harvest at time zero and subsequently at 15-day intervals for about 80 days. The harvested plant material was separated into component parts (leaf, stem, crown and root), oven dried at 80°C and weighed. Crown in each tiller represented a 5 cm long segment (from the point of rooting upwards) in *C. gayana* and *P. coloratum* and a corresponding 3 cm long segment in *L. perenne* and *P. pratensis*. The significance of separating crown lies in the fact that these basal parts of grass tillers often serve as storage organs. In this paper shoot weight in *L. perenne* and *P. pratensis* was the sum of cumulative leaf weight and crown weight, while the same in *C. gayana* and *P. coloratum* was the sum of cumulative weights of leaf and stem plus the weight of crown. The weight of the material clipped between the sampling dates s_1 and s_2 was added to the shoot weight for the sampling date s_2 . Relative growth rates were calculated following Evans (1972).

TNC of shoots, roots and crowns were determined following Smith (1969). The nitrogen content was determined for shoots only, following Piper (1944). Protein content was then calculated by multiplying N content by 6.25. Chemical analysis of plant material was done at I, IV and VI harvests. Each analysis was replicated thrice. For chemical analysis, shoot refers to leaves in *L. perenne* and *P. pratensis* and to leaves + stem in *C. gayana* and *P. coloratum*.

3. Results and discussion

3.1 Plant biomass

Total biomass tended to increase with time attaining the highest value at final harvest (figure 1). Water stress had an unfavourable effect on it in all cases. Generally, the

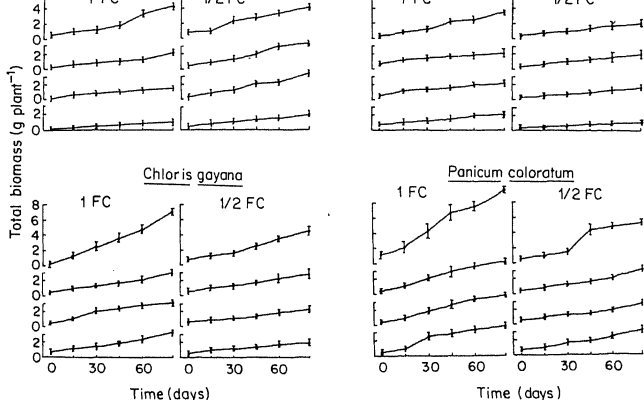


Figure 1. Cumulative dry matter yield of whole plant of four grass species subjected to various clipping treatments under two soil water conditions. In each case the curves from top to bottom represent respectively; unclipped, monthly clipped, fortnightly clipped and weekly clipped plants. Vertical bars represent ± 2 SE (total length of bar = 4 SE).

reduction in biomass was maximum for weekly clipped plants followed by fortnightly clipped, monthly clipped and unclipped plants.

Table 1 indicates the degree to which water stress affected the cumulative total biomass and biomass of different organs. The adverse effect of water stress on total biomass of unclipped, monthly clipped and fortnightly clipped plants was comparatively greater in magnitude in two C_4 species followed by *P. pratensis*, while in weekly clipped plants, the maximum reduction occurred in *C. gayana* followed by *P. coloratum*. *L. perenne* either showed least reduction in total biomass (unclipped) or increased biomass (all clipping regimes) under $\frac{1}{2}$ FC. Water stressed, monthly clipped plants of *P. pratensis* also accumulated greater biomass on the final harvest compared to 1 FC. This suggests that the two C_4 species were more severely affected by water stress than the two C_3 species. Similar results were obtained by Carrol (1943) and Boyer (1970).

Shoot biomass, in general, was most adversely affected by water stress in *P. coloratum* while the root biomass was most adversely affected in this species and in *P. pratensis* (table 1). In *L. perenne*, with the exception of unclipped plants, the root biomass was higher under $\frac{1}{2}$ FC. This was perhaps due to some stimulation of root growth by slight moisture stress as has been reported for certain other species (Eaton 1942; Jarvis 1963).

Table 2 shows the adverse effect of clipping on biomass. The reduction in biomass in all organs became sharper with increasing clipping frequency, particularly in *L. perenne* and *P. coloratum*. In the two C_3 species the reduction in biomass due to clipping was greater under 1 FC compared to $\frac{1}{2}$ FC, while in the C_4 species the reduction was almost

Percent clipping regime, due to water stress. Values are calculated as $\frac{1 \text{ FC} - \frac{1}{2} \text{ FC}}{1 \text{ FC}} \times 100$.

Species	Clipping treatment	Total biomass	Shoot	Root
<i>L. perenne</i>	Weekly clipped	+ 159	+ 116	+ 586
	Fortnightly clipped	+ 82	+ 56	+ 258
	Monthly clipped	+ 54	+ 46	+ 88
	Unclipped	11	5	18
<i>P. pratensis</i>	Weekly clipped	51	53	40
	Fortnightly clipped	27	22	58
	Monthly clipped	+ 2	3	+ 44
	Unclipped	41	29	58
<i>C. gayana</i>	Weekly clipped	55	50	+ 38
	Fortnightly clipped	35	42	+ 93
	Monthly clipped	8	11	+ 16
	Unclipped	39	45	+ 7
<i>P. coloratum</i>	Weekly clipped	39	41	15
	Fortnightly clipped	51	51	44
	Monthly clipped	43	46	37
	Unclipped	48	46	54

The effect of species and treatments (water level and clipping) was significant ($P < 0.01$) in all cases.

Table 2. Per cent reduction or increase (values prefixed with + sign) in biomass of different organs in the 4 species under two water conditions, due to clipping frequency. Values are calculated as $\frac{\text{Unclipped} - \text{clipped}}{\text{Unclipped}} \times 100$.

Species	Clipping treatment	1 FC			$\frac{1}{2}$ FC		
		Total biomass	Shoot	Root	Total biomass	Shoot	Root
<i>L. perenne</i>	Weekly clipped	82	71	96	48	44	69
	Fortnightly clipped	65	45	90	29	10	56
	Monthly clipped	50	27	79	13	+ 14	52
<i>P. pratensis</i>	Weekly clipped	44	18	85	50	46	75
	Fortnightly clipped	42	22	75	30	24	75
	Monthly clipped	44	21	82	5	+ 7	37
<i>C. gayana</i>	Weekly clipped	53	50	78	58	54	73
	Fortnightly clipped	60	56	83	57	53	69
	Monthly clipped	59	58	63	47	33	61
<i>P. coloratum</i>	Weekly clipped	46	35	82	37	28	67
	Fortnightly clipped	43	31	81	47	25	77
	Monthly clipped	32	20	69	26	17	58

clipping followed by *C. gayana*, *P. pratensis* and *P. coloratum*. In contrast, under $\frac{1}{2}$ FC the C_3 plants were more resistant to clipping compared to C_4 plants. In all 4 species clipping affected the root system more adversely compared to shoot system. It was suggested that frequent clipping and consequent recovery allows little time for the manufacture of surplus photosynthate, hence downward translocation is limited. This results in the reduction of root growth (Bokhari and Singh 1974). Detling *et al* (1979) and Painter and Detling (1981) also observed that increased frequency of clipping reduces root yield.

In all 4 species under both water conditions the root: shoot ratio (R:S ratio) of unclipped plants was generally higher than of clipped ones during most of the experimental period (figure 2). In *L. perenne* and *C. gayana* (also in *P. pratensis* except for the last 3 samplings) water stress resulted in a relatively greater accumulation of dry matter in roots. In *P. coloratum* the effect of water stress on R:S ratio was not marked. However, at most harvests the clipped plants had a higher R:S ratio under $\frac{1}{2}$ FC. Several workers reported an increase in R:S ratio under water stress (Black 1968; Struk and Bray 1970). According to Loomis *et al* (1971), the water stress slows shoot growth more and sooner than it does root growth. Increasing clipping frequency under both water conditions tended to reduce the R:S ratio. Evidently clipping suppressed root growth more than the shoot growth.

Unclipped C_3 plants had a greater R:S ratio compared to the unclipped C_4 plants (figure 2). The higher R:S ratio is thought to be conducive to drought tolerance (Maximov 1929; Parker 1968).

3.2 Net production

The mean values for net production (cumulative dry weight at the final harvest minus the initial dry weight) are given in table 3. In *L. perenne* the total net production of water stressed plants (in all clipping treatments) was greater than in 1 FC. In contrast in other species (monthly clipped plants of *P. pratensis* being an exception) it decreased due to water stress.

Generally, net production decreased by increasing clipping frequency (table 3). Compared to the unclipped plants, the clipped plants of all 4 species under 1 FC exhibited lower net production. The root production was comparatively more adversely affected by clipping compared to the shoot production. In the water stressed C_3 plants the reducing effect of clipping was of a smaller magnitude. In contrast, in the two C_4 species, generally, the clipping frequency affected net production to the same degree under both water conditions. For example, total net production of weekly and fortnightly clipped plants in *C. gayana* under 1 FC was reduced by 62 % and 65 % and under $\frac{1}{2}$ FC by 61 % and 64 % respectively.

3.3 Relative growth rate

The relative growth rate on shoot (R_sGR) and root (R_rGR) in all species fluctuated considerably with no consistent temporal pattern except for the fact that in majority of cases the rate declined towards the end of the experimental period.

The mean values of R_sGR (table 4) indicate that the water stress had an adverse effect on the two C_4 species. In the two C_3 species the water stress tended to induce the R_sGR

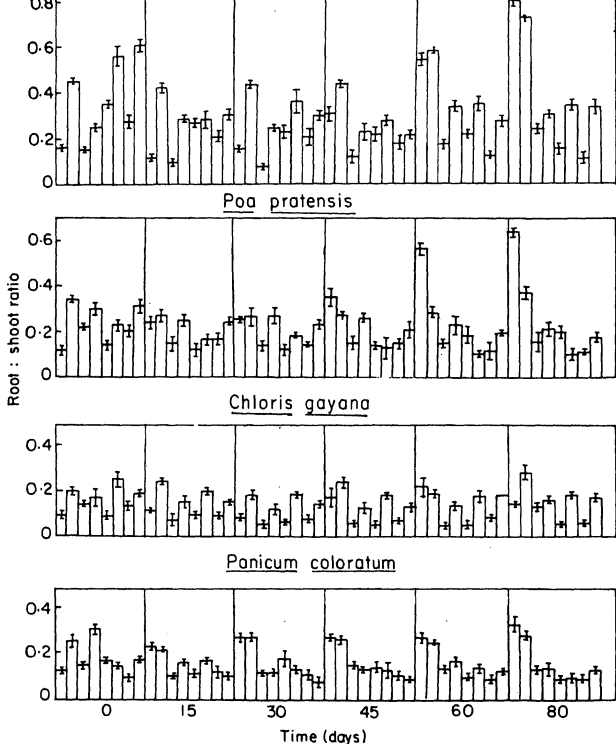


Figure 2. Root: shoot ratio of four grass species subjected to various clipping treatments under two soil water conditions. For each harvest, the bars from left to right represent: unclipped under 1 FC, unclipped under $\frac{1}{2}$ FC, monthly clipped under 1 FC; monthly clipped under $\frac{1}{2}$ FC, fortnightly clipped under 1 FC, fortnightly clipped under $\frac{1}{2}$ FC, weekly clipped under 1 FC and weekly clipped under $\frac{1}{2}$ FC. Vertical bars represent ± 1 SE (total length of bar = 2 SE).

Table 3. Net production of different species during the experimental period under different treatments (g plant⁻¹).

	1 FC			½FC		
	Shoot	Root	Total	Shoot	Root	Total
<i>L. perenne</i>						
Weekly clipped	0.48	0.02	0.50	1.17	0.31	1.48
Fortnightly clipped	1.04	0.10	1.14	1.70	0.52	2.22
Monthly clipped	1.38	0.35	1.73	2.10	0.65	2.75
Unclipped	1.83	1.82	3.65	1.75	1.34	3.06
<i>P. pratensis</i>						
Weekly clipped	1.19	0.08	1.27	0.53	0.04	0.57
Fortnightly clipped	1.14	0.26	1.40	1.06	0.08	1.14
Monthly clipped	1.27	0.15	1.42	1.39	0.28	1.67
Unclipped	1.79	1.32	3.11	1.24	0.46	1.70
<i>C. gayana</i>						
Weekly clipped	2.49	0.11	2.60	1.17	0.18	1.35
Fortnightly clipped	2.23	0.11	2.34	1.08	0.17	1.25
Monthly clipped	2.16	0.26	2.42	1.78	0.28	2.06
Unclipped	5.85	0.84	6.69	2.65	0.80	3.45
<i>P. coloratum</i>						
Weekly clipped	4.31	0.39	4.70	2.37	0.28	2.65
Fortnightly clipped	4.72	0.41	5.13	2.01	0.19	2.20
Monthly clipped	5.57	0.70	6.27	2.97	0.36	3.33
Unclipped	6.43	2.33	8.76	3.53	1.08	4.61

except for unclipped plants of both species, and weekly clipped plants of *P. pratensis*. The effect of clipping was not consistent and marked.

Water stress had a stimulatory effect on the mean R_RGR of all clipped plants of *L. perenne* and in weekly clipped plants of *C. gayana* (table 4). In other plant treatments it reduced the R_RGR . Increasing clipping frequency also reduced the mean R_RGR in all cases.

In general, the $R_SGR : R_RGR$ ratio indicated that clipping shifted the balance between shoot and root growth so as to favour the former more strongly. This is in conformity with the observations of other workers such as Kleinendorst and Brouwer (1969) and Davies (1974). This tendency was more strongly realised under 1 FC and more markedly in *L. perenne* and *C. gayana*. Although there was no equanimity in the response of different components to water stress, the RGR at the whole plant level (R_WGR) was reduced except for clipped plants of *L. perenne* and the fortnightly and monthly clipped plants of *P. pratensis*, in the latter cases water stress stimulated the R_WGR (table 4). Thus growth rates of the C_4 plants were more severely affected by water stress. Clipping treatments generally reduced the R_WGR under both water conditions except for *P. coloratum*, where the effect of clipping was not consistent.

3.4 Total non-structural carbohydrate

Table 4. Effect of water stress and clipping on relative growth rates (R_sGR , R_rGR and R_wGR) (as mean across all sampling intervals) of four grass species.

Species/clipping treatment	Mean relative growth rate of shoot (R_sGR ; $g\ g^{-1}\ day^{-1}$)		Mean relative growth rate of root (R_rGR ; $g\ g^{-1}\ day^{-1}$)		Mean relative growth rate of whole plant (R_wGR ; $g\ g^{-1}\ day^{-1}$)	
	1 FC	‡FC	1 FC	‡FC	1 FC	‡FC
<i>Perenne</i>						
Weekly clipped	0.016 ± 0.005	0.021 ± 0.010	0.004 ± 0.001	0.013 ± 0.003	0.014 ± 0.004	0.019 ± 0.006
Fortnightly clipped	0.022 ± 0.010	0.026 ± 0.009	0.011 ± 0.003	0.019 ± 0.005	0.020 ± 0.009	0.024 ± 0.007
Monthly clipped	0.020 ± 0.006	0.024 ± 0.005	0.025 ± 0.006	0.027 ± 0.009	0.015 ± 0.005	0.020 ± 0.005
Unclipped	0.021 ± 0.008	0.020 ± 0.011	0.040 ± 0.009	0.025 ± 0.007	0.026 ± 0.007	0.022 ± 0.010
<i>pratiensis</i>						
Weekly clipped	0.012 ± 0.004	0.012 ± 0.002	0.007 ± 0.003	0.005 ± 0.003	0.013 ± 0.004	0.011 ± 0.002
Fortnightly clipped	0.014 ± 0.006	0.020 ± 0.005	0.018 ± 0.004	0.010 ± 0.002	0.015 ± 0.006	0.019 ± 0.004
Monthly clipped	0.017 ± 0.008	0.023 ± 0.005	0.012 ± 0.004	0.019 ± 0.006	0.016 ± 0.008	0.022 ± 0.006
Unclipped	0.021 ± 0.008	0.019 ± 0.006	0.042 ± 0.012	0.021 ± 0.004	0.028 ± 0.007	0.021 ± 0.006
<i>rayana</i>						
Weekly clipped	0.021 ± 0.005	0.019 ± 0.009	0.011 ± 0.004	0.017 ± 0.005	0.018 ± 0.004	0.019 ± 0.009
Fortnightly clipped	0.024 ± 0.011	0.016 ± 0.006	0.017 ± 0.009	0.012 ± 0.005	0.023 ± 0.010	0.015 ± 0.006
Monthly clipped	0.024 ± 0.006	0.020 ± 0.006	0.018 ± 0.007	0.018 ± 0.005	0.023 ± 0.006	0.019 ± 0.006
Unclipped	0.041 ± 0.016	0.021 ± 0.004	0.049 ± 0.010	0.024 ± 0.009	0.041 ± 0.015	0.021 ± 0.007
<i>oloratum</i>						
Weekly clipped	0.028 ± 0.012	0.022 ± 0.007	0.029 ± 0.015	0.015 ± 0.004	0.028 ± 0.010	0.021 ± 0.006
Fortnightly clipped	0.034 ± 0.012	0.019 ± 0.005	0.029 ± 0.012	0.016 ± 0.008	0.033 ± 0.011	0.019 ± 0.005
Monthly clipped	0.035 ± 0.011	0.026 ± 0.005	0.033 ± 0.010	0.017 ± 0.007	0.035 ± 0.012	0.024 ± 0.004
Unclipped	0.028 ± 0.008	0.026 ± 0.010	0.042 ± 0.014	0.038 ± 0.017	0.034 ± 0.009	0.028 ± 0.012

effect of species and treatments (water level and clipping) was significant at $P < 0.05$ in all cases.

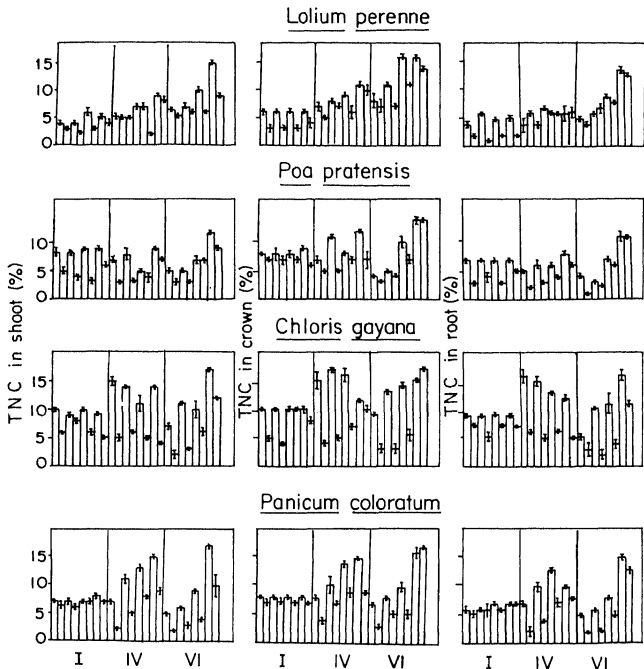


Figure 3. TNC content of different organs of 4 species subjected to various clipping treatments under two soil water conditions. For each harvest the bars from left to right represent: weekly clipped under 1 FC, weekly clipped under $\frac{1}{2}$ FC, fortnightly clipped under 1 FC, fortnightly clipped under $\frac{1}{2}$ FC, monthly clipped under 1 FC, monthly clipped under $\frac{1}{2}$ FC, unclipped under 1 FC and unclipped under $\frac{1}{2}$ FC. Vertical bars represent ± 1 SE (total length of bar = 2 SE).

The clipping treatments interfered with this general pattern and the clipped plants did not have as much opportunity to replenish their reserve by the end of the growth cycle as the unclipped plants. Under 1 FC root, shoot and crowns of clipped plants of the two C_4 species exhibited an initial increase in the TNC content followed by a decline. Similar trend was observed in big sagebrush (*Artemisia tridentata*) by Coyne and Cook (1970). In other cases such as root and shoots of monthly clipped plants of *P. pratensis* under $\frac{1}{2}$ FC the content declined initially and therefore there were no replenished

TNC content tended to decline rapidly with time.

Table 5 indicates that generally, water stress adversely affected the TNC content of different organs. The decline in TNC content due to water stress was much more marked in the two C_4 species than in the two native C_3 species and in severely clipped plants than in moderately clipped plants. The effect of water stress on carbohydrate reserves depends upon the species involved, the growth stage and the environmental factors (Bokhari and Dyer 1974). Perhaps a lesser decrease in TNC due to water stress in C_3 compared to C_4 plants indicates greater adaptability of the former to water stress conditions.

Clipping also caused a reduction in the TNC level (table 5). The reduction was greater in severely clipped compared to moderately clipped plants. Clipping caused more reduction in the TNC levels of water stressed plants. Generally, again the water stressed C_4 plants suffered the adverse effect of clipping on TNC more than the water stressed C_3 plants. The level of reduction due to severe clipping was greater for crowns and roots than for shoots. Evidently the amount of assimilates produced by the remaining leaf

Table 5. Effect of water stress (per cent decrease in TNC content relative to 1 FC value at the final harvest) and clipping (per cent decrease in TNC content relative to unclipped value at the final harvest) on TNC accumulation of 4 grass species.

Species/ clipping treatment	Effect of clipping								
	Effect of water stress			1 FC			$\frac{1}{2}$ FC		
	Shoot	Crown	Root	Shoot	Crown	Root	Shoot	Crown	Root
<i>L. perenne</i>									
Weekly clipped	15	17	26	57	50	62	42	53	70
Fortnightly clipped	14	35	0	53	31	60	36	49	50
Monthly clipped	40	28	15	34	2	35	36	20	40
Unclipped	37	12	7	—	—	—	—	—	—
<i>P. pratensis</i>									
Weekly clipped	43	39	76	61	70	62	69	81	91
Fortnightly clipped	27	20	25	62	66	71	62	72	77
Monthly clipped	6	29	21	43	28	35	26	47	45
Unclipped	28	4	5	—	—	—	—	—	—
<i>C. gayana</i>									
Weekly clipped	73	68	49	60	42	66	83	86	75
Fortnightly clipped	77	81	78	36	11	33	77	87	78
Monthly clipped	42	60	63	46	8	29	52	71	62
Unclipped	35	0	34	—	—	—	—	—	—
<i>P. coloratum</i>									
Weekly clipped	57	57	60	72	54	69	77	88	86
Fortnightly clipped	53	30	65	68	51	62	74	69	86
Monthly clipped	56	44	40	52	40	49	63	71	65
Unclipped	42	0	14	—	—	—	—	—	—

TNC content in different organs of 4 species varied significantly with time, water level and clipping frequency ($P < 0.01$).

Perry and Chapman (1974) and Ogden and Loomis (1972) also indicated a severe depletion of carbohydrate reserves following clipping in basin wild rye (*Elymus cinereus*) and intermediate wheatgrass (*Agropyron* sp.), respectively.

Generally, crowns had greatest TNC content in all 4 species in this investigation. Sosebes and Wiebe (1971) believed that increased accumulation of reserves in perennating organs such as crowns might be an effective survival mechanism, allowing plants to utilize reserves for increased growth when environmental conditions improved.

3.5 Crude protein

Protein content in shoots of 4 grass species under two soil water conditions and various clipping regimes is given in figure 4. Water stress also had a depressing effect on protein content of shoots in all four species (table 6). Bonner (1950) argued that water stress may induce proteolysis in leaves. Protein hydrolysis has also been reported in other plants subjected to water stress (Kemble and Macpherson 1954; Parker 1969).

Mall and Singh (1977) reported that increasing clipping frequency increased the protein content of *Themeda triandra*. According to them, frequent clipping which prevents plants from becoming overmature, increases the protein content at the expense of yield. In the present study also protein content in shoots of all 4 species increased in clipped plants (table 6). This increase was pronounced in the severely clipped compared to the moderately clipped plants. Further, the water stressed plants subjected to severe clipping accumulated more protein compared to the water stressed unclipped plants. The behaviour of protein content was thus reverse of TNC content. Besides increasing the protein content, clipping also resulted in greater relative leaf weight in mature plants. In table 7 the mean leaf weight as per cent of total plant dry weight in unclipped and weekly clipped plants of the 4 grasses are compared at final harvest. It is evident that the proportion of leaves was much more in the clipped plants compared to the unclipped plants. Thus the plants which underwent defoliations had relatively more leafy material rich in protein. Thus clipping also increases the potential growth period of the plant if no other factor is limiting because of the stimulated new leaf initiation and growth (Bokhari and Singh 1974).

4. Conclusions

It may be concluded from the above that the overall performance of C_3 species was much superior compared to C_4 plants. The present C_3 species were shown to develop a greater amount of water saturation deficit (wsd) and showed a comparatively greater amount of leaf rolling under conditions of water stress compared to C_4 plants (Pande and Singh 1981). The two C_3 plants were thus able to withstand the stress of water and clipping in a much better way than the C_4 plants. These results are in contrast to the general belief that the C_4 species have a greater drought resistance than C_3 species (see Singh *et al* 1980). Perhaps the C_4 plants possess a competitive advantage over C_3 plants only under conditions of high temperature and intermittent water stress (Doliner and Jolliffe 1979), while in a low temperature region at high altitudes, such as Naini Tal,

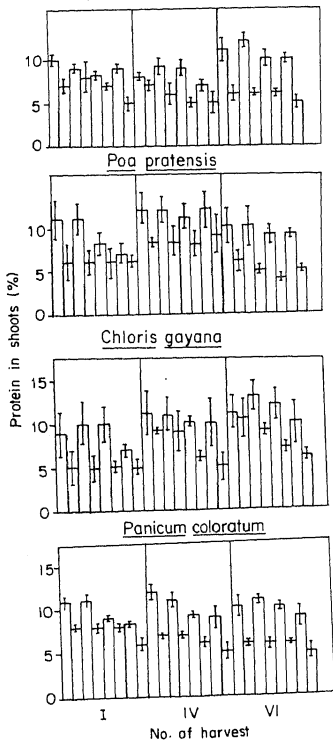


Figure 4. Protein content of shoots of 4 grass species subjected to various clipping treatments under two soil water conditions. For rest of the explanation see figure 3.

the C_3 plants are better at resisting drought. Sims and Singh (1983) found for the North American grasslands that communities dominated by cool season species (C_3) were comparable to or more efficient in energy capture and water use than the communities dominated by warm season (C_4) plants.

Table 6. Effect of water stress (per cent decrease in protein content relative to corresponding 1 FC value at the final harvest) and clipping (per cent increase in protein content relative to unclipped value at the final harvest) on protein accumulation in shoots of 4 grass species

Species/clipping treatment	Effect of water stress	Effect of clipping	
		1 FC	$\frac{1}{2}$ FC
<i>L. perenne</i>			
Weekly clipped	43	7	31
Fortnightly clipped	49	8	31
Monthly clipped	41	0	23
Unclipped	54	—	—
<i>P. pratensis</i>			
Weekly clipped	38	12	40
Fortnightly clipped	47	8	16
Monthly clipped	59	0	0
Unclipped	51	—	—
<i>C. gayana</i>			
Weekly clipped	6	14	87
Fortnightly clipped	28	28	62
Monthly clipped	39	21	33
Unclipped	43	—	—
<i>P. coloratum</i>			
Weekly clipped	44	15	30
Fortnightly clipped	47	15	23
Monthly clipped	42	11	30
Unclipped	50	—	—

Total protein in shoots varied significantly in all 4 species with water level, clipping frequency and water level \times clipping frequency interactions (all at $P < 0.01$)

Table 7. Leaf dry weight as per cent of total plant dry weight at final harvest.

Species	1 FC		$\frac{1}{2}$ FC	
	Weekly clipped	Unclipped	Weekly clipped	Unclipped
<i>L. perenne</i>	80	44	66	47
<i>P. pratensis</i>	83	44	73	49
<i>C. gayana</i>	71	48	52	37
<i>P. coloratum</i>	72	43	73	50

References

Bassiri A, Kosh-Khui M and Rovhani I 1977 The influence of simulated moisture stress conditions and osmotic substrates on germination and growth of cultivated and wild safflowers; *J. Agric. Sci.* **88** 95–100

- Bokhari U G and Singh J S 1974 Effect of temperature and clipping on growth, carbohydrate reserves and root exudation of Western wheatgrass in hydroponic culture; *Crop Sci.* **14** 790-794
- Bonner J 1950 *Plant Biochemistry* (New York: Academic Press) p. 537
- Boyer J S 1970 Differing sensitivity of photosynthesis to low leaf water potentials in Corn and Soybean; *Plant Physiol.* **46** 236-239
- Brouwer R and de Wit C T 1969 A simulation model of plant growth with special attention to root growth and its consequences; in *Root Growth* (ed.) W J Whittington (London: Butterworth) pp. 224-244
- Brown R H and Blasser R E 1965 Relationship between reserve carbohydrate accumulation and growth rate in orchard grass (*Dactylis glomerata* L.) and tall fescue (*Festuca arundinacea*); *Crop Sci.* **5** 577-582
- Carroll J C 1943 Effects of drought, temperature and nitrogen on turf grasses; *Plant Physiol.* **18** 19-36
- Coyne P I and Cook C W 1970 Seasonal carbohydrate reserve cycle in eight desert range species; *J. Range Manage.* **23** 438-444
- Davies A 1974 Leaf tissue remaining after cutting and regrowth in perennial ryegrass; *J. Agric. Sci. Camb.* **82** 165-172
- De Puit J E and Caldwell M M 1975 Gas exchange of three cool semi-desert species in relation to temperature and water stress; *J. Ecol.* **63** 835-856
- Detling J K, Dyer M I and Winn D 1979 Net photosynthesis, root respiration and regrowth of *Bouteloua gracilis* following simulated grazing; *Oecologia (Berlin)* **41** 127-134
- Dolliner L H and Jolliffe P A 1979 Ecological evidence concerning the adaptive significance of the C-4 dicarboxylic acid pathway of photosynthesis; *Oecologia (Berlin)* **38** 23-34
- Eaton F M 1942 Toxicity and accumulation of chloride and sulphate salts in plants; *Agric. Res.* **64** 357-399
- Evans G C 1972 *The quantitative analysis of plant growth*. (Oxford, London, Edinburgh, Melbourne: Blackwell Scientific Publications)
- Jameson D A 1963 Responses of individual plants to harvesting; *Bot. Rev.* **29** 532-594
- Jarvis M S 1963 A comparison between the water relations of species with contrasting type of geographical distribution in British Isles; in *The water relations of plants* (eds) A J Rutter and F H Whitehead (Oxford: Blackwell Scientific Publication) pp 289-312
- Kemble V and Macpherson H T 1954 Liberation of aminoacid in perennial grass during wilting; *Biochem. J.* **58** 46-49
- Kleinendorst A and Brouwer R 1969 *Growth responses of two clones of perennial ryegrass to excision of roots or shoots*, Instituut voor Biologisch en Schekunding Onderzoek Van Landbouwgewassen, Wageningen, Jaarboek. 19-25
- Loomis R S, Williams W A and Hall A D 1971 Agricultural Productivity; *Ann. Rev. Plant Physiol.* **22** 431-468
- Mall L P and Singh V P 1977 Responses of *Themeda triandra* Forsk grass to various clipping treatments; *J. Indian Bot. Soc.* **56** 202-207
- Maximov N A 1929 *The plant in relation to water* (London: Allen and Unwin)
- Mooney H A 1972 The carbon balance of plants; *Ann. Rev. Ecol. Syst.* **3** 315-346
- Ogden P R and Loomis W E 1972 Carbohydrate reserves of intermediate wheatgrass after clipping and etiolation treatments; *J. Range Manage.* **25** 29-32
- Painter E L and Detling J E 1981 Effects of defoliation on net photosynthesis and regrowth of Western wheatgrass; *J. Range Manage.* **34** 68-71
- Pande H and Singh J S 1981 Comparative biomass and water status of four grass species grown under two soil water conditions; *J. Range Manage.* **34** 480-484
- Pande U and Singh J S 1980 A quantitative study of the forest floor, litter fall and nutrient return in an oak-conifer forest in Himalaya. I. Composition and dynamics of forest; *Acta Oecol.* **2** 49-61
- Parker J 1968 Drought resistance mechanism; in *water deficits and plant growth* (New York: Academic Press) pp 195-234
- Parker J 1969 Further studies of drought resistance in woody plants; *Bot. Rev.* **35** 317-370
- Perry L J and Chapman S R 1974 Effects of clipping on carbohydrate reserves in basin wildrye; *Agron. J.* **66** 67-69
- Piper C S 1944 *Soil and Plant Analysis* (New York: Interscience Publishers Inc.)
- Sims P L and Singh J S 1978 The structure and function of ten western North American grasslands; III. Net primary production, turnover and efficiencies of energy capture and water use; *J. Ecol.* **66** 573-597
- Singh J S, Trlica M J, Risser P G, Redmann R E and Marshall J K 1980 Auto-trophic subsystem; in

- Grasslands, *Systems Analysis and Man* (eds) A I Breymeyer and G M van Dyne (London: Cambridge University Press) pp 59–200
- Smith D 1969 *Removing and analysing total non-structural carbohydrates from plant tissues*; Res. Rep. No 41, Research Division, University of Wisconsin, Madison
- Sosebee R E and Wiebe H H 1971 Effect of water stress and clipping on photosynthate translocation in two grasses; *Agron. J.* **64** 14–17
- Struck G J and Bray J R 1970 Root ratios of native forest herbs and *Zea mays* at different soil moisture levels; *Ecology* **51** 892–893
- Thorne G N 1960 Variation with age in net assimilation rate and other growth attributes of sugar beet, potato and barley in a controlled environment; *Ann. Bot.* 356–371
- Trlica M J and Cook C W 1972 Carbohydrate reserves of crested wheatgrass and Russian Wildrye as affected by development and defoliation; *J. Range Manage.* **25** 430–435
- Trlica M J and Singh J S 1979 Translocation of assimilates and creation, distribution utilization of reserves; in *Arid-land ecosystem: Structure, Functioning and Management* (eds) R A Perry and D W Goodall (London: Cambridge University Press) Vol. 1, IBP 16, pp. 537–571
- Yegappan T M, Paton D M, Gates C T and Muller W J 1982 Water stress in sunflower (*Helianthus annuus* L.)
1. Effect on plant development; *Ann. Bot.* **46** 61–70

Activity of isoproturon on leaf cell membrane permeability

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Abstract. Disruption of leaf cell membranes was studied after the foliar application of isoproturon [N-(4-isopropyl phenyl)-N,N-dimethyl urea] through the parameters of electrolyte leakage and the quantity of efflux in ions. Differential activity of isoproturon, on leaf cell membranes of *Sida acuta*, *Cleome viscosa* and *Amaranthus viridis* was observed. A high degree of correlation of resistance or susceptibility of a particular plant to the herbicide with appropriate change in electrolyte leakage and efflux of ions was observed.

Keywords. Isoproturon; electrolyte leakage; ion efflux; cell membrane permeability.

1. Introduction

Experimental evidence showing the effects of herbicides especially on the permeability of leaf-cell membranes *per se* is limited. Several chemicals dalapon, 2,4,5-T, ansar, amitrole and paraquat were shown to affect membrane permeability of leaves (Mary *et al* 1979; Umapathy *et al* 1979). Leakage of electrolytes as measured by the changes in the conductivity of the ambient medium is a convenient and accurate method to study the effects of several herbicides on leaf cell membrane permeability.

2. Materials and methods

Healthy young seedlings (20 days old) of *Amaranthus viridis* L, *Sida acuta* Burn, *Cleome viscosa* Linn, were transplanted to experimental plots from their natural habitat and grown under natural photoperiod (28°C/20°C night). Twenty days after transplantation, 500 mg l⁻¹ concentration of isoproturon, [N-(4-isopropyl phenyl)-N,N-dimethyl urea] without any wetting agent was applied as a foliar spray up to the drip point at 8:00 AM with a manually operated 'Aspee' sprayer. Control plants were maintained by simultaneous spray of deionised water.

Matured leaves (third from the apex) with similar symptoms were excised at one day interval up to 4 days after herbicide treatment. The electrolytic conductivity of the ambient medium in which the leaf discs were incubated was measured following the method of Vanstone and Stobbe (1977), with slight modifications, using Philips Conductivity Bridge (Model PA 9500/50). The medium in which the leaf discs were immersed was directly fed to flame photometer to determine the quantity of potassium efflux present in the leachates.

3. Results

Data presented in table 1 reveal that the electrolytic conductivity increased steadily both in the case of *S. acuta* and *C. viscosa*, indicating greater damage to the leaf cell

Plant species	Control	1	2	3	4	5	6
<i>A. viridis</i>	22.46 ± 1.10	30.40 ± 1.21	36.68 ± 1.20	34.25 ± 1.14	30.85 ± 1.04	28.23 ± 1.04	26.16 ± 1.01
<i>S. acuta</i>	17.85 ± 0.92	25.44 ± 0.86	29.65 ± 0.94	33.35 ± 1.01	38.98 ± 1.12	39.10 ± 1.02	39.33 ± 0.94
<i>C. viscosa</i>	21.26 ± 1.12	31.45 ± 1.20	36.25 ± 1.16	40.86 ± 1.18	45.20 ± 1.85	45.98 ± 0.64	46.19 ± 1.02

Values are mean of 5 observations ± SE

Table 2. Effect of isoproturon on potassium content of leachates of leaves ($\text{mg dm}^{-2} \text{h}^{-1}$)

Plant species	Isoproturon						
	Days after treatment						
	Control	1	2	3	4	5	6
<i>A. viridis</i>	0.3126 ± 0.015	0.4562 ± 0.019	0.5142 ± 0.017	0.4806 ± 0.021	0.4325 ± 0.016	0.4116 ± 0.026	0.3986 ± 0.016
<i>S. acuta</i>	0.2845 ± 0.012	0.4165 ± 0.016	0.4721 ± 0.026	0.5126 ± 0.022	0.6046 ± 0.019	0.6076 ± 0.014	0.6098 ± 0.12
<i>C. viscosa</i>	0.4126 ± 0.018	0.6003 ± 0.014	0.6935 ± 0.021	0.7829 ± 0.015	0.8552 ± 0.016	0.8610 ± 0.013	0.8629 ± 0.020

Values are mean of 5 observations ± SE

membranes. Consequently, the increase in quantity of potassium efflux in both *S. acuta* and *C. viscosa* was well correlated with the increased electrolytic conductivity (table 2). Wilting of leaves both in the case of *S. acuta* and *C. viscosa* was observed after herbicidal spray indicating their susceptibility. In contrast the initial increase and final recovery in electrolytic conductivity and potassium efflux was observed in the case of *A. viridis* indicating the resistance of the plant to that herbicide at that particular concentration. The increase in electrolytic leakage and potassium efflux was rapid, particularly in *S. acuta* and *C. viscosa*, and preceded visual injury symptoms caused by the contact activity of the herbicide.

4. Discussion

It has been reported that many herbicides including phenyl ureas interfere with lipid synthesis (Rivera and Penner 1979). In the present study, the continuous rise in electrolytic conductivity and concomitant increase in leakage of potassium ions from

followed by final recovery in electrolytic conductivity and potassium efflux may be due to the uptake of ions from intercellular spaces. It can therefore be concluded that the change in cell membrane permeability is an effective determinant for the resistant or susceptible nature of the plants for a given herbicide. It is also evident from the study that one of the initial effects of isoproturon on leaves is increased cell membrane permeability.

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References

- Mary C, Brien O and Prendeville G N 1979 Effect of herbicides on cell membrane permeability in *Lemna minor*; *Weed Res.* **19** 331-334
- Umapathy P, Swamy P M and Das V S R 1979 Herbicidal influence on the leakage of electrolytes from the leaves of scrub weeds; *Indian J. Exp. Biol.* **17** 816-817
- Vanstone D E and Stobbe E H 1977 Electrolytic conductivity a rapid measure of herbicidal injury; *Weed Sci.* **25** 352-354
- Rivera C M and Penner D 1979 Effect of herbicides on plant cell membrane lipids; *Residue Rev.* **70** 45-76

Leaf-litter decomposition in arid to semi-arid climatic conditions

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Abstract. Decomposition of 3 leaf litter species, viz., *Datura innoxia*, *Solanum sysimbrifolium* and mixed grass were studied. Within 150 days, about 90% of *Datura*, 66% of *Solanum* and 51% of mixed grass litter disappeared. Mean relative decomposition rate was higher during rainy season for all the 3 litter species. From the stepwise multiple regression analysis it was concluded that in arid to semi-arid locality decomposition process is mainly controlled by soil moisture.

Keywords. Decomposition; *Datura innoxia*; *Solanum sysimbrifolium*; mean relative decomposition rate; stepwise multiple regression.

1. Introduction

In terrestrial ecosystems, the dead organic matter plays an important role as an energy source for microorganisms and also as a carrier of nutrients to soil. Because of its importance in nutrient cycling and in supporting the saprophagic component of ecosystems, decomposition has gained paramount importance in recent times. Thus, detrital ecology has ushered into the lime light and an understanding of decomposition rates of various plant species, the effect of weather variables, the pattern of nutrient release and the role of microorganisms have assumed key positions.

Arid to semi-arid regions are characterized by high temperature and low rainfall, where the rainfall is not only meagre but highly erratic in pattern, subjecting the organisms to climatic stress conditions. The aim of the present study was to investigate the temporal pattern of decomposition, the differences in decomposition rates of different litter species and relationships with some weather variables.

2. Materials and methods

2.1 The study area

The study was conducted at Rajkot (N 20° 58' and E 70° 20') in a grazing land ecosystem, dominated by *Dichanthium annulatum* and *Aristida royleana*. *Datura innoxia* and *Solanum sysimbrifolium* occupy special niches in the ecosystem. The soils are silty-clayey loams derived from Basalt (Deccan trap). The overall range of variation in chemical characters was: pH 7.2–8.3, total nitrogen 0.014–0.018%, organic carbon 2.5–4.3%, available phosphorus 0.0006–0.0034%, potassium 0.0048–0.0064% and calcium 0.5–1.5%.

2.2 Climate

The mean annual precipitation at Rajkot is 675 mm. The mean maximum temperature ranges from 36–44°C and the mean minimum from 7.5–23.5°C. The year is divisible

2.3 Litter decomposition

For the decomposition study, newly senesced leaves of *Datura innoxia*, *Solanum sysimbrifolium* and mixed grass litter were collected. The mixed grass litter included *Aristida royleana*, *Dichanthium annulatum* and *Melanocenchris jacquemontii*. These samples were air dried. Litter bags of galvanized wire netting (15 × 10 cm) with 1 mm mesh size were used and 5.0 g of litter were kept in bags separately. Moisture content was determined for each litter stock prior to placing the litter bags in the field. The litter bags were randomly placed on the floor on 16 June 1980. Sampling was done at fortnightly intervals. Three litter bags of each litter type were retrieved randomly on each sampling date. The material from individual bags was washed with a fine jet of water through a 100 μ mesh. The washed material was then oven-dried at 80°C to constant weight. The soil temperature and moisture contents were determined on each sampling date. Soil water content is expressed as per cent of oven-dried soil, after Miskin (1968).

The rate of CO₂ evolution from decomposing litter was determined *in situ* by the inverted box method (Witkamp 1966).

3. Results

3.1 Weight loss pattern

The rate of decomposition was measured as loss of dry weight. The time series data on weight loss for the 3 litter types are presented in figure 1. The per cent cumulative weight

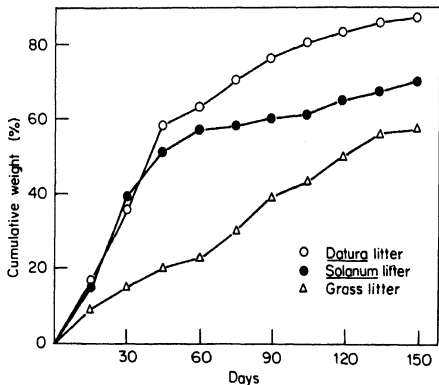


Figure 1. Cumulative weight loss of the 3 types of litters.

loss in the 3 litter types indicates considerable differences. Significant temporal variations were also observed among the litter types. Among the 3 different litters, *Datura* decomposed fastest followed by *Solanum* and mixed grass litter. Analysis of variance indicated statistically significant differences due to time ($P < 0.01$), species ($P < 0.01$) and the interaction of species \times time ($P < 0.01$). Within 45 days of commencement of experiment, about 60% of *Datura*, 50% of *Solanum* and 20% of mixed grass litter disappeared. In all 3 litter types, weight loss was maximum during rainy season. In the 3 litters, after an initial rapid loss, the decomposition rates decreased and about 90% of *Datura*, 66% of *Solanum* and 51% of mixed grass litter disappeared within 150 days.

The instantaneous rates of decomposition was calculated as per cent weight loss per day. The highest rate of decomposition was 1.09, 1.00 and 0.53% per day observed during the period of 2 July to 1 August for *Datura*, *Solanum* and mixed grass litter, respectively. The relation between weight loss pattern and time was tested through regression analysis (figure 2). The resulting regression equations were:

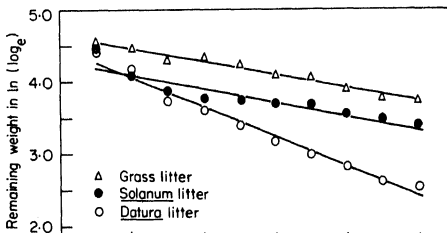
Datura innoxia litter $Y = 3.294 + 0.010 X$ ($r^2 = 0.847$, $P < 0.01$). *Solanum sysimbrifolium* litter $Y = 3.211 + 0.008 X$ ($r^2 = 0.770$, $P < 0.01$). Mixed grass litter $Y = 2.250 + 0.010 X$ ($r^2 = 0.820$, $P < 0.01$). Where Y = per cent weight loss in \log_e and X = time interval (days)

3.2 Mean relative decomposition rate (R)

In order to understand the effect of weather variables on decomposition rate, mean relative decomposition rates (R) were calculated for different species using the following modified expression of Gupta and Singh (1981):

$$R = \frac{\log_e W_0 - \log_e W}{t_0 - t_1}$$

Where R is mean relative decomposition rate ($\text{g g}^{-1} \text{ day}^{-1}$), W is weight at time t_1 (g) and W_0 is weight at time t_0 (g). The R values for all the 3 litter species are given in table 1.



Sampling dates	R in g/g/day		
	<i>Datura</i> litter	<i>Solanum</i> litter	Mixed grass litter
02.07.1980	0.012	0.011	0.006
17.07.1980	0.017	0.022	0.005
01.08.1980	0.029	0.015	0.004
16.08.1980	0.008	0.009	0.003
31.08.1980	0.014	0.002	0.007
15.09.1980	0.015	0.003	0.008
30.09.1980	0.012	0.001	0.005
16.10.1980	0.011	0.008	0.009
01.11.1980	0.010	0.005	0.008
16.11.1980	0.008	0.005	0.002
For total study period	0.014	0.008	0.006

The highest R value was obtained for *Datura* litter ($0.029 \text{ g g}^{-1} \text{ day}^{-1}$) recorded during the third sampling interval. The mean R values for the entire study period were 0.014, 0.008 and $0.006 \text{ g g}^{-1} \text{ day}^{-1}$ for *Datura*, *Solanum* and mixed grass litter, respectively. The mean relative decomposition rates were higher during rainy season in all 3 litter types.

3.3 Interrelationships between weight loss and some biotic and abiotic variables

In order to understand the factors which govern the process of decomposition, both simple and multiple regression analyses were performed considering weight loss as dependent variable and following as independent variables:

- litter moisture (in %)
- soil moisture (in %)
- soil temperature (in $^{\circ}\text{C}$)
- CO_2 evolution from litter (in $\text{mg m}^{-2} \text{ hr}^{-1}$)
- number of bacteria in litter ($\times 10^8$)
- number of actinomycetes in litter ($\times 10^8$)
- number of fungi in litter ($\times 10^8$).

The result of simple regression analysis is presented in table 2. The correlation between weight loss and soil moisture in all the 3 litter species was highly significant ($P < 0.01$) and explained about 65–80% variability. The relationship of weight loss of *Datura* litter was not significant with litter moisture, CO_2 evolution, number of bacteria, number of actinomycetes and number of fungi in litters. The relationships between weight loss in grass litter and soil temperature or CO_2 evolution were significant ($P < 0.05$).

The equations of stepwise multiple regression along with R^2 values and significance level are given in table 3. Among the 7 independent variables studied, soil moisture

Y	X	r	a	b	P
Weight loss in <i>Datura</i> litter	Soil moisture content	0.991	00.946	5.314	***
	Litter moisture content	0.105	41.708	0.942	NS
	Soil temperature	0.548	26.765	0.888	*
	CO ₂ evolution from litter	0.122	9.032	-0.171	NS
	No. of bacteria in litter	0.374	5.456	-0.101	NS
	No. of actinomycetes	0.124	1.164	0.112	NS
	No. of fungi in litter	0.069	0.608	0.036	NS
Weight loss in <i>Solanum</i> litter	Soil moisture content	0.982	-6.922	7.474	***
	Litter moisture content	0.179	26.140	2.799	NS
	Soil temperature	0.439	25.997	1.009	NS
	CO ₂ evolution from litter	0.489	10.430	-0.804	NS
	No. of bacteria in litter	0.173	3.746	-0.535	NS
	No. of actinomycetes	0.130	2.306	-0.330	NS
	No. of fungi in litter	0.120	2.189	0.117	NS
Weight loss in mixed grass litter	Soil moisture content	0.894	-13.993	6.623	***
	Litter moisture content	0.413	32.034	-0.985	NS
	Soil temperature	0.599	49.283	-4.377	**
	CO ₂ evolution from litter	0.627	13.697	-1.239	**
	No. of bacteria in litter	0.175	7.024	-0.831	NS
	No. of actinomycetes	0.001	0.712	0.001	NS
	No. of fungi in litter	0.292	-0.023	0.146	NS

NS, Non-significant; * Significant at $P < 0.1$; ** Significant at $P < 0.05$; *** Significant at $P < 0.01$.

Table 3. Stepwise multiple regression equations of 3 litters along with R^2 values and significance level.

Dependent variable	Regression equation	R^2	Significance level
<i>Datura</i> litter	$Y = -0.143 + 0.184 x_2$	0.981	***
<i>Solanum</i> litter	$Y = 1.230 - 0.010 x_1 + 0.136 x_2$	0.988	***
Mixed grass	$Y = 2.453 + 0.148 x_2 - 0.695 x_7$	0.879	***

***Significant at $P < 0.01$; x_1 , Litter moisture (in %); x_2 , Soil moisture (in %); x_3 , Soil temperature (in °C); x_4 , CO₂ evolution from litter (in mg/m²/hr); x_5 , No. of bacteria in litter (in $\times 10^6$); x_6 , No. of Actinomycetes in litter (in $\times 10^8$); x_7 , No. of fungi in litter (in $\times 10^8$).

emerged as the most important factor controlling the decomposition process under arid to semi-arid conditions.

4. Discussion

The decomposition rate of leaf litter of *Datura innoxia*, *Solanum sysimbrifolium* and mixed grass varied between 0.37–0.14 % per day. Gupta and Singh (1981) and Rajvanshi and Gupta (1980) reported similar rates of decomposition for *Desmostachya bipinnata*,

percentage decrease in later stages indicates optimum conditions in rainy season. The high rates of decomposition correspond with high moisture content of soil. The effect of moisture, promoting the microbial growth may explain the increased rate of weight loss during the rainy season. Van der Drift (1963), Karenlampi (1971) and Rochow (1974) have emphasized that moisture is more important than temperature in litter decomposition. In the present study also, weight loss of the 3 litter types yield statistically significant relationship with moisture, whereas, with soil temperature, the relationship was not significant.

Stepwise multiple regression analysis also depicted the predominance of soil moisture over other variables on litter decomposition. Thus, it can be concluded that in arid to semi-arid locality, decomposition would be mainly controlled by soil moisture.

The differences in weight loss among *Datura*, *Solanum* and mixed grass litter can be attributed to the amount of nitrogen and physical nature of the plant material. Initial nitrogen content in *Datura*, *Solanum* and mixed grass litter was 2.5, 1.0 and 0.85%, respectively. The higher weight loss in *Datura* was mainly due to its high nitrogen content. The differences in decomposition rates between *Solanum* and mixed grass litter can be attributed to the differences in the physical nature of plant material, as the nitrogen content did not differ much. The mixed grass litter was more resistant to leaching resulting in slower rate of decomposition. These findings corroborate with the earlier investigations of Edwards (1977), Gupta and Singh (1981) and Pink *et al* (1950). Differences among plant material in overall decomposition rate is a function of differences in the relative proportions of labile and recalcitrant fractions initially present in each type of plant material. A significant relationship was also found between the relative initial proportion of labile to recalcitrant fractions and both initial lignin content and initial C/N ratio (Wieder and Lang 1982). Cromack and Monk (1975), Fogal and Cromack (1977), Meentemeyer (1970) and Melillo *et al* (1982) have suggested that initial lignin content or initial C/N ratio may be a reasonable predictor of the rate of decomposition. However, Hunt (1977) suggested that the decomposition is dependent upon the shift in the relative proportion of labile to recalcitrant fractions. Thus, under uniform conditions, the process of decomposition is very similar over a wide variety of plant materials despite considerable overall differences in decomposition rates.

References

- Cromack K and Monk C D 1975 *Litter production, decomposition and nutrient cycling in a mixed hardwood watershed and a white pine watershed, in mineral cycling in southeastern ecosystems* (eds) F G Howell, J B Gentry and M H Smith (United States Energy Research and Development Administration, Symposium Series CONF-740513, Washington, DC, USA) pp 609-624
- Edwards P J 1977 Studies of mineral cycling in a montane Rain forest in New Guinea. I. Distribution of organic matter in the vegetation and soil; *J. Ecol.* **65** 943-969
- Fogal R and Cromack K Jr 1977 Effect of habitat and substrate quality on Douglas fir litter decomposition in Western Oregon; *Can. J. Bot.* **55** 1632-1640
- Gupta S R and Singh J S 1981 The effect of plant species, weather variables and chemical composition of plant material on decomposition in a tropical grassland; *Plant Soil* **59** 99-117
- Hunt H W 1977 A simulation model for decomposition in grasslands; *Ecology* **58** 469-484
- Karenlampi L 1971 Weight loss of leaf litter on forest soil surface in relation to weather at kevo station, Finnis Lapland; *Rep. Kevo Subarct. Res. Stn.* **8** 101-103

- Meentemeyer V 1978 Macroclimate and lignin control of litter decomposition rates; *Ecology* **59** 465–472
- Melillo J M, Aber J D and Muratore J F 1982 Nitrogen and lignin control of hardwood leaf litter decomposition dynamics; *Ecology* **63** 621–626
- Misra R 1968 *Ecology Workbook*, (New Delhi: Oxford and IBH Publishing Co.) p 244
- Pink L A, Allison F E and Sherman M S 1950 Maintenance of soil organic matter. II. Losses of carbon and nitrogen from young and mature plant materials during decomposition in soil; *Soil Sci.* **69** 391–401
- Rajvanshi R and Gupta S R 1980 Decomposition of litter in a tropical dry deciduous forest; *Int. J. Ecol. Environ. Sci.* **6** 37–49
- Rochow J J 1974 Litter fall relations in a Missouri Forest; *Oikos* **25** 80–85
- Van der Drift J 1963 The disappearance of litter in mull and moor in connection with weather conditions and the activity of the macrofauna; in *Soil organisms* (eds) J Deeksen and J Van der Drift (Amsterdam: North Holland Publ. Co.) pp 125–133
- Wieder R K and Lang G E 1982 A critique of the analytical methods used in examining decomposition data obtained from litter bags; *Ecology* **63** 1636–1642
- Witkamp M 1966 Decomposition of leaf litter in relation to environmental conditions, microflora and microbial respiration; *Ecology* **47** 194–201

Acquired cycloheximide resistance in *Neurospora crassa* and *Sclerotium rolsfii*

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Abstract. Acquired resistance to the antibiotic cycloheximide developed by *Neurospora crassa* and *Sclerotium rolsfii* was studied. Both the fungi gained certain level of tolerance to the antibiotic just after a single exposure and by serial transfers could adapt to the several-fold initial inhibitory dosage. Cycloheximide-resistance in both the cases was not a stable characteristic and was lost gradually on serial transfers in fungicide-free media. The resistant strains of both the fungi showed poor growth and decreased sporulation or sclerotia formation in fungicide-free media. *Sclerotium rolsfii* developed cross-resistance to Difolatan but not to Polyoxin-D, Hinosan and Bayleton. The cycloheximide-resistance in *Sclerotium rolsfii* was probably due to the conversion of cycloheximide into isocycloheximide which is a less toxic structural analog, as revealed by thin layer chromatographic studies of culture filtrates of resistant and sensitive strains. The resistant strain of *Sclerotium rolsfii* retained its pathogenicity to tomato, mustard and chilli seedlings. However, the loss of sclerotia forming capacity and the instability of the acquired resistance trait may prove to be of ecological disadvantage to the resistant strain.

Keywords. Acquired resistance; cycloheximide; *Neurospora*; *Sclerotium*.

1. Introduction

Cycloheximide (= Actidione) is a broad spectrum antifungal antibiotic isolated from *Streptomyces griseus* (Ford and Leach 1948). It is generally toxic to eukaryotes and is useful as a fungicide only when the host plant is less sensitive than the pathogen. However, it is widely used as a biochemical tool to block protein synthesis in eukaryotes. Though cycloheximide (CH) was considered as a specific inhibitor of protein synthesis, it has recently been shown to inhibit ribosomal RNA synthesis (Timberlake and Griffin 1974) and DNA synthesis (Sullia and Griffin 1977) in *Achlya bisexualis*. In view of the importance of CH, the present study on acquired resistance to CH in *Sclerotium rolsfii* and *Neurospora crassa* has been conducted. Earlier investigations have shown that some fungi gain resistance to CH, the extent of acquired resistance depending on the species (Salkin 1975; Griffin *et al* 1978; Sullia 1979). The mechanism of acquired resistance to CH has not been thoroughly understood. Although some work has been done with yeasts, filamentous fungi have scarcely been studied from the point of view of resistance.

2. Materials and methods

2.1

The test fungi were (i) *Neurospora crassa* Shear and Dodge-culture from Bangalore University Culture Collection No. 186, a saprobe (ii) *Sclerotium rolsfii* Sacc.-isolated

2.2 Radial growth of test fungi in CH-amended medium

The test fungi were grown in PDA amended with CH ranging from 0.25–60 µg/ml and radial growth measurement technique was used for assessment of growth at 30°C. The ED₅₀ value was determined by plotting the dosage-response curve. The level of resistance acquired due to first exposure to 0.5 µg/ml (*N. crassa*) and 40 µg/ml (*S. rolfii*) was tested by a second transfer to CH-amended media. For other experiments, resistant strains were obtained by 7 serial subcultures in CH-amended medium.

2.3 Determination of maximum concentration of antibiotic tolerated

This was determined by serially subculturing the fungi in increasing concentrations of CH i.e., 1.5, 2.0, 2.5 and 3.0 µg/ml for *N. crassa* and 60, 80, 120, 160 and 200 µg/ml for *S. rolfii*.

2.4 Testing the stability of acquired resistance

The CH-resistant strains of *N. crassa* and *S. rolfii* were transferred to fungicide-free media through 7 subcultures and after each transfer the test organisms were reexposed to 0.5 and 40 µg/ml CH, respectively to check retention or loss of acquired resistance. The growth and sporulation of resistant strains were compared with those of sensitive strains, when returned to fungicide-free media and transferred to fungicide-free medium for 3 successive subcultures.

2.5 Cross-tolerance studies

Cross-resistance studies were conducted with *S. rolfii* alone. Cross-resistance of CH-resistant strain towards Hinosan (5 µg/ml), Polyoxin-D (400 µg/ml), Difolatan (1,000 µg/ml) and Bayleton (200 µg/ml), was studied on amended PDA. The plates were incubated at 30°C for 20 days and colony diameters measured periodically.

2.6 Assay of CH in culture filtrates of sensitive and resistant strains

To determine the comparative ability of sensitive and resistant strains to convert CH into other compounds, mainly analogs of CH, the fungal culture filtrate after a definite period of growth was subjected to analysis by thin-layer chromatography (TLC) method detailed below. The CH-resistant and sensitive strains of *S. rolfii* were transferred to replicates of 250 ml flasks containing 50 ml of PD-broth each and incubated at 28°C in a rotary shaker adjusted to 120 rpm. CH was introduced into each flask after 5 days of uniform mycelial growth in the flasks to obtain a concentration of 40 µg/ml.

Mycelial mats were filtered off with Whatman filter paper after 15 days. The culture filtrate was extracted with equal volume of chloroform and the chloroform layer was separated, evaporated to dryness and the residue redissolved in 1 ml of chloroform. This solution was used for chromatographic assay. Silica gel TLC plates were used for

Name of compound	<i>R_f</i> values	
	Ethyl acetate	Ethyl ether
CH	0.75	0.38
Iso-CH	0.80	0.67
CH-acetate	0.80	0.42
Streptimidone	0.58	0.38
CH-oxime	0.16	0.00
Streptovitacin-A	0.23	0.00
Anhydro-CH	0.80	0.52

spotting and the chromatograms were run with ethyl acetate and ethyl ether upto a distance of 8–10 cm. The plates were scanned under uv light for fluorescence and the spots were marked with a pencil. *R_f* values were calculated.

The *R_f* values of pure samples of CH and its analogs were determined on silica gel thin layer plates separately with ethyl ether and ethyl acetate. The analogs of CH used were Streptimidone, Streptovitacin-A, Iso-CH, CH-acetate, Anhydro-CH and CH-oxime (table 1).

The objective of the experiment was to determine whether the fungus had the ability to convert CH into any of the known structural analogs which were less toxic to fungi.

2.7 Assessment of the virulence of the resistant strains

2.7.1 Pre-emergence damping-off: Samples of sterilised soil were mixed with mycelial bits of resistant and sensitive strains of *S. rolfisii* and incubated for 48 hr. After 48 hr of incubation, seeds of tomato (*Lycopersicon esculentum* Mill.), chilli (*Capsicum annum* L.) and mustard (*Brassica nigra* L. (Koch)) were sown. Observations were made on the 10th day for tomato and mustard and on the 15th day for chilli. The percentage mortality was calculated based on 3 replicate pots.

2.7.2 Post-emergence damping-off: 10-Day old seedlings of tomato and mustard and 15-day old seedlings of chilli in pots were treated with inocula of resistant and sensitive strains of *S. rolfisii* fed to soil. Seedling mortality was recorded on the 20th day for tomato and mustard and on the 25th day for chilli.

3. Results and discussion

3.1 Sensitive and resistant strains

N. crassa was highly sensitive to CH, the ED₅₀ value being 0.5 µg/ml. Figure 1 shows that in the fungicide-free medium (control) the growth curves of both sensitive and resistant isolates were identical and steeply rising showing no lag phase. A longer lag phase and a flattening of the growth curve was evident with increasing concentration of antibiotic

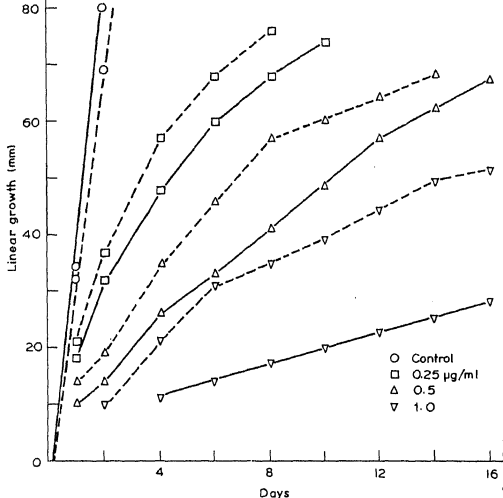


Figure 1. Linear growth of resistant and sensitive isolates of *N. crassa* in different concentrations of cycloheximide. Resistant isolate was priorly exposed to 0.5 µg/ml CH.

and this was more pronounced in the case of sensitive strains which showed much slower growth in all the 3 concentrations viz., 0.25, 0.5 and 1.0 µg/ml. On the 16th day the resistant isolate showed 45 mm colony diameter in 1.0 µg/ml compared to 25 mm of sensitive isolate. The adaptation of the fungus due to a single exposure to the antibiotic is evident from the experiment.

S. rolfii was less sensitive to CH than *N. crassa*, the ED₅₀ value being 20 µg/ml. The adaptation of the fungus to CH is quite evident on comparison of growth curves of sensitive and resistant isolates on medium containing 40 and 60 µg/ml CH (figure 2). At 60 µg/ml CH, the resistant isolate showed a steep growth curve, the fungus reaching a colony diameter of 65 mm in 8 days by which time there was no discernible growth of sensitive isolate. The resistance from single exposure was indeed very striking. Figure 2 also shows that the sensitive isolate plated on 60 µg/ml, after a prolonged lag phase slowly picked up growth and the curve showed an upward trend towards the end showing gain in tolerance during the prolonged maintenance of the fungus in amended medium. Adaptation of the fungus due to a single exposure has been reported in several species of fungi (Salkin 1975). Griffin *et al* (1978) have shown acquired resistance to CH in *Achlya* and they have also shown that some other fungi (e.g., *Cladosporium*) possess

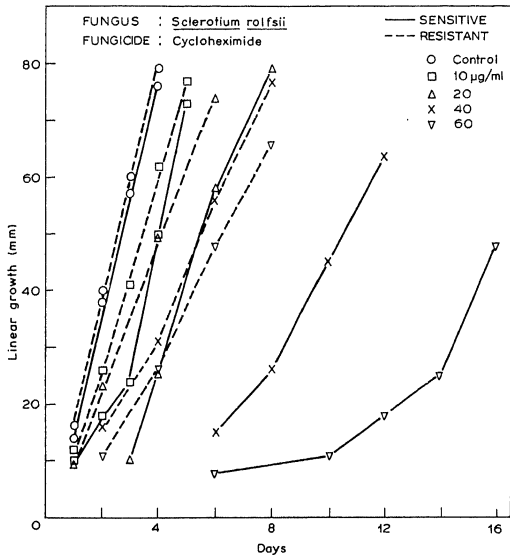


Figure 2. Linear growth of resistant and sensitive isolates of *S. rolfsii* in different concentrations of CH. Resistant isolate was priorly exposed to 40 µg/ml CH.

built-in resistance to CH. In the present study, *S. rolfsii* showed similar 'built-in' or 'constitutive resistance' to CH.

3.2 Antibiotic level

Through serial transfers from 1.5 µg/ml to 2.0, 2.5 and 3.0 µg/ml CH, *N. crassa* could be 'trained' to tolerate the antibiotic to some extent. The maximum concentration of the antibiotic to which the fungus could be trained was 2.5 µg/ml i.e., 5 times the ED₅₀ dosage. Beyond that there was no discernible growth. Saprobic fungi are known to be more sensitive to CH than zoopathogenic fungi (Salkin 1975; Griffin *et al* 1978; Sullia 1979), and *N. crassa* is mainly a saprobic fungus.

The CH-resistant isolate of *S. rolfsii* was transferred from 60 µg/ml to 80, 120, 160 and 200 µg/ml CH. The sensitive isolate could not grow beyond 80 µg/ml. The trained isolate could grow at concentration upto 200 µg/ml. However, the resistant isolate produced no sclerotia beyond 100 µg/ml. Dharma Vir and Sharma (1980) have reported

<i>N. crassa</i> (8th day)								<i>S. rolfssii</i> (6th day)							
Serial nos of transfers in fungicide-free medium								Serial nos of transfers in fungicide-free medium							
Cl.	1	2	3	4	5	6	7	Cl.	1	2	3	4	5	6	7
20	50	58	60	45	29	21	21	16	55	39	37	32	25	22	18

Cl, Control (unadapted)

1-7, Resistant strain after each transfer to fungicide-free medium.

3.3 Stability of acquired resistance

From table 2 it is clear that the resistant isolate of *N. crassa* retained its 'acquired resistance' to some extent during 5 serial transfers in fungicide-free medium. However, there was rapid loss of acquired resistance after the 6th transfer showing that acquired CH resistance is not a stable characteristic.

Somewhat similar result was obtained with *S. rolfssii* resistant to CH, where the acquired resistance was lost gradually (table 2). Ashida (1965) has shown that acquired resistance of *Sclerotinia fructicola* to copper and mercury was retained for 5 transfers but was completely lost by the tenth. Loss of CH-resistance has also been reported in certain saprobic and phytopathogenic fungi by Salkin (1975). When returned to fungicide-free medium there was reversion to initial levels of sensitivity, indicating that resistance to CH was not a genetic process but a purely transitory biochemical phenomenon. Grover *et al* (1961), showed in *Sclerotinia fructicola* and *S. laxa* that Actidione (= CH) resistance did not involve genetic selection and so the adapted isolates reverted back to parent types after growing for 2-5 generations in CH-free media.

3.4 Growth of resistant strains

The resistant strain of *N. crassa* on transfer to fungicide-free medium showed poor growth and no perithecia formation (table 3). This characteristic manifested for 3 successive transfers.

Resistant strain of *S. rolfssii* showed slower growth and poor yield of sclerotia compared to parental isolate in fungicide-free media (table 3). Dekker and Gielink (1979) demonstrated in laboratory tests that increased resistance to Pimaricin in selected strains of *Cladosporium cucumerinum* and *Fusarium oxysporum* f.sp. *narcissi* was associated with decreased radial growth and sporulation in vitro. Pimaricin, however, is an antifungal antibiotic chemically unrelated to CH. This shows that loss of sporulating ability in fungi may be induced by a large number of antifungal agents irrespective of their mode of action.

3.5 Cross-resistance

CH-resistant strain of *S. rolfssii* showed negative cross-resistance phenomenon towards Polyoxin-D and Hinosan as the sensitive strain fared better than resistant in media

during 3 successive transfers.

	Linear growth at 48 hr (in mm)	Ave. no. of perithecia/ sq. cm area after 7 days	Ave. no. of sclerotia/ plate after 7 days	
	<i>N. crassa</i>	<i>S. rolsii</i>	<i>N. crassa</i>	<i>S. rolsii</i>
S	89	38	84	39
R	13	33	0	315

S, CH sensitive; R, CH resistant.

Table 4. Cross-resistance of CH-resistant strain of *S. rolsii* to other antifungal agents.

Incubation time (days)	Fungal strain	Radial growth (mm)				
		CH	D	H	DF	B
5	S	10	20	56	5	8
	R	68	14	40	19	10
10	S	45	30	80	5	11
	R	80	26	66	59	17
15	S	*	53	*	5	15
	R	*	48	*	66	22
20	S	*	70	*	5	31
	R	*	64	*	80	36

CH, Cycloheximide; D, polyoxin-D; H, hinosan; DF, Difolatan; B, Bayleton; S, CH sensitive; R, CH resistant.

* Plates fully covered by fungal growth.

amended with these fungicides (table 4). In Difolatan, the CH-resistant strain covered 80 mm diameter on 20th day, whereas the sensitive isolate showed no growth indicating cross-resistance. There was also some degree of cross-resistance to Bayleton though not to the same extent as to Difolatan (figure 3). Even though, there was cross-resistance to Difolatan, there was no sclerotia formation. CH is an antibiotic which inhibits protein synthesis, whereas Difolatan interferes with the process of decarboxylation. The two obviously differ in their modes of action. Cross-resistance between totally unrelated compounds is relatively rare. Jurkowska (1962) showed cross-resistance to Zinc and Nickel salts in *Aspergillus niger* tolerant to copper sulphate.

3.6 Conversion of antibiotic by resistant strain

The extract of the culture filtrate of the CH-resistant strain in *S. rolsii* showed one prominent spot in the chromatogram developed with solvent ethyl acetate (figure 4) which is identified as isocycloheximide (by referring to the R_f value given in table 1), which is less toxic than CH. Cycloheximide inhibits protein synthesis, DNA synthesis and

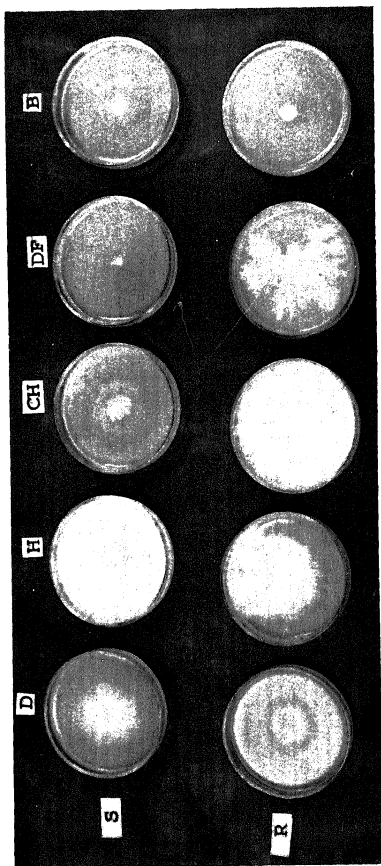


Figure 3. Cross-resistance of CH-resistant strain of *S. roffsii* to different antifungal agents. D, Polyoxin-D; H, Hinosan; CH, Cycloheximide; DF, Difolatan; B, Bayleton; R, Resistant strain; S, Sensitive strain.

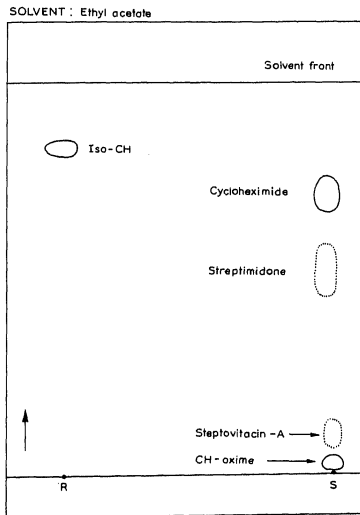


Figure 4. Diagram showing silicagel thin layer chromatogram of chloroform extracts of culture filtrates of CH-resistant and sensitive strains of *S. rolsii* developed in ethyl acetate. The spots were marked by observing the fluorescence under UV light.

ribosomal RNA synthesis, whereas Iso-CH inhibits only one of the processes (Griffin 1981). The culture filtrate of sensitive strain of *S. rolsii* showed several spots in addition to CH (figures 4 and 5). The spots were identified as Streptimidone, Streptovitacin-A and CH-oxime by comparing their R_f values with table 1. Isocycloheximide was present as a very faint spot. The sensitive strain retained most of the CH in the original form which is toxic to the fungus. The resistant isolate presumably metabolised almost all the CH into Iso-CH. Thus detoxification through conversion of the fungicide into a less toxic analog seems to be the main reason for resistance in this case. Somewhat similar results of conversion of a chemical by a fungus, but to a more toxic compound instead of less, has been reported. Gasztonyi and Josepovitis (1979) reported this with reference to the uptake and metabolism of triadimefon by fungi. A highly fungitoxic product (triadimenol) was formed to a high level in sensitive fungi, but it was very low or not found in resistant fungi.

3.7 Pathogenicity

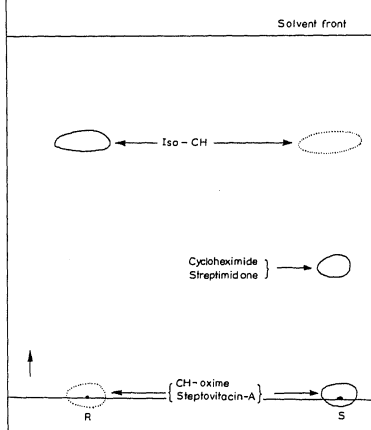


Figure 5. Diagram showing TLC of chloroform extracts of culture filtrates of CH-resistant and sensitive strains of *S. rolfsii* developed in ethyl ether. The spots were scanned through UV light.

Table 5. Pathogenicity of cycloheximide-resistant and sensitive strains of *S. rolfsii* to chilli, mustard and tomato seedlings (average of 3 replicates).

Host	Fungal strain	Pre-emergence damping off			Post-emergence damping off		
		Ave. no. of seeds sown/pot	Ave. no. of seeds emerged/pot	Percentage mortality	Ave. no. of seedlings initially present/pot	Ave. no. of seedlings dead/pot	Percentage of mortality
Chilli	Resistant	20	11.6	41.1 ^a	14.4	4.4	30.4 ^b
	Sensitive	20	13.0	35.0	16.6	3.3	19.9
Mustard	Resistant	20	8.6	56.6 ^b	9.3	4.3	46.4 ^b
	Sensitive	20	12.3	38.3	12.6	3.8	30.2
Tomato	Resistant	20	13.9	30.3 ^a	18.6	5.3	28.5 ^b
	Sensitive	20	14.9	25.3	17.6	2.9	16.9

Difference between resistant and sensitive significant at 1% level as indicated by 't' test.

^a Significant at 5% level.

even more virulent than the original strain in the post-emergence stage. It has already been stated that the CH-resistant isolate lost its resistance to the antibiotic and its capacity to produce sclerotial bodies when transferred repeatedly to CH-free medium. This shows that though the fungus has not lost its virulence in the process of adaptation, it would, nevertheless, suffer from a disadvantage in competition as the adaptation is not a stable characteristic. The examples of fungi losing their acquired resistance or the phenomenon of reversion can be found in literature e.g., Kasugamycin resistance in *Pyricularia oryzae* (Miura *et al* 1975), Polyoxin-resistance in *Alternaria kikuchiana* (Kohmoto *et al* 1974) and benomyl-resistance in *Cercospora beticola* (Ruppel and Scott 1974).

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References

- Ashida J 1965 Adaptation of fungi to metal toxicants; *Annu. Rev. Phytopathol.* **3** 153-174
- Dekker J and Gielink A J 1979 Acquired resistance to pimarinin in *Cladosporium cucumerinum* and *Fusarium oxysporum* f. sp. *narcissi* associated with decreased virulence; *Neth. J. Plant Pathol.* **85** 67-73
- Dharma Vir and Sharma R K 1980 Adaptation of *Sclerotium rolfsii* to systemic fungicide; *Curr. Sci.* **49** 285-286
- Ford J H and Leach B E 1948 Actidione an antibiotic from *Streptomyces griseus*; *J. Am. Chem. Soc.* **70** 1223-1225
- Gasztonyi M and Josepovits G 1979 The activation of triadimefon and its role in the selectivity of fungicide action; *Pestic. Sci.* **10** 57-65
- Griffin D H 1981 *Fungal Physiology*, (New York: John Wiley and Sons) p 383
- Griffin D H, Sullia S B and Salkin I F 1978 Resistance of selected saprobic and zoopathogenic fungi to Cycloheximide; *J. Gen. Microbiol.* **105** 127-134
- Grover R K and Duain Moore J 1961 Adaptation of *Sclerotinia fructicola* and *Sclerotinia laxa* to higher concentration of fungicides; *Phytopathology* **51** 399-401
- Jurkowska H 1962 Investigation on the adaptability of *Aspergillus niger* to copper; *Bull. Ind. Acad. Gracove. Ser. B.* **4** 167-201
- Kohmoto K, Miyake H, Nishimura S and Udagawa H 1974 Distribution and chronological population shift of Polyoxin resistant strains of black spot fungi of Japanese pear, *Alternaria kikuchiana* in field; *Ann. Phytopathol. Soc. Jpn.* **40** 220
- Miura H, Ito H and Takahashi S 1975 Occurrence of resistant strains of *Pyricularia oryzae* to kasugamycin as a cause of the diminished fungicidal activity to rice blast; *Ann. Phytopathol. Soc. Jpn.* **41** 415-417
- Ruppel E G and Scott P R 1974 Strains of *Cercospora beticola* resistant to benomyl in the USA; *Plant Dis. Rep.* **58** 434-436
- Salkin I F 1975 Adaptation of Cycloheximide; in vitro studies with filamentous fungi; *Can. J. Microbiol.* **21** 1413-1419
- Sullia S B and Griffin D H 1977 Inhibition of DNA synthesis by Cycloheximide and Blasticidin-S is independent of their effect on protein synthesis; *Biochim. Biophys. Acta* **475** 14-22
- Sullia S B 1979 Acquired tolerance to antifungal agents in fungi; *Pestology* **3** 10-13
- Timberlake W E and Griffin D H 1974 Differential effects of Cycloheximide and other inhibitors of protein synthesis on in vivo ribosomal RNA synthesis in *Achlya bisexualis*; *Biochim. Biophys. Acta* **353** 248-252

Ontogeny of palmately compound leaves in angiosperms:

1. *Tabebuia pentaphylla* Hense

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Abstract. The palmately compound leaf of *Tabebuia pentaphylla* is initiated by periclinal division in the hypodermal layer at the flank of the maximal shoot apex, which lacks cytohistological zonation. The growth of the leaf primordium is diffuse until it reaches a height of 50–60 μm after which an adaxial meristem makes it conspicuously thick at the basal region. The first pair of lateral leaflets arise about 50 μm below the tip of the leaf primordium when the latter is about 150 μm high and before the differentiation of a well defined marginal meristem. The second pair of leaflets arises subsequently below the first. The terminal portion becomes the central leaflet. The sites of leaflet initiation are the terminal endings of acropetally differentiating procambial strands. The 6-layered plate meristem of the leaflet lamina arises from a marginal meristem whose submarginal initial is wedge shaped. Leaflet venation is comptodromous and the ultimate areoles lack free vein endings.

Keywords. *Tabebuia pentaphylla*; leaf ontogeny; palmately compound leaf; shoot apex; dicot leaf ontogeny.

1. Introduction

The leaf is morphologically prominent and physiologically the most important part of the angiosperms. Several features in which the monocotyledons differ from the dicotyledons, leaf character is an important one, the former exhibiting a parallel venation and the latter a reticulate venation in general. Apart from this, while compound leaves of various types are found in the dicotyledons, most of the monocotyledons are simple leaved and the compound leaf is an exception, occurring only in some members of the Liliaceae, Araceae, Taccaceae and Dioscoreaceae. In Arecaceae the adult leaf is pinnately compound in many species but it has been shown (Eames 1953; Periasamy 1965, 1967) that ontogenetically all palm leaves are simple and the compound nature arises due to disconnections in the plicated lamina during the late stages of ontogeny. The leaves of Cyclanthaceae are ontogenetically similar to those of Arecaceae (Wilder 1976, 1981).

The special attributes that characterise the monocotyledonous leaves have led to morphological interpretations like the phyllode theory (Henslow 1911; Arber 1918), leaf base theory (Knoll 1948; Kaplan 1970a) and the unifacial concept (Knoll 1948; Roth 1949; Thielke 1948), all of which aim at interpreting the monocot leaves in terms of the dicot leaves and to establish morphological difference between them. Difference of opinion also exists with regard to the phylogenetic relation between the simple and the compound leaf (Eames 1961; Sporne 1974). Furthermore, it is not known whether in view of the envisaged morphological differences between the dicot and monocot

assess how far the morphological interpretations of the angiosperm leaf are in accord with the little known ontogenetic picture of the palmately compound lamina. An overall discussion of the picture that has emerged from this study will be presented in the final paper of the series.

2. Materials and methods

Shoot apices with leaves at various stages of development were collected from the campuses of St. Joseph's College, Tiruchirapalli and Presidency College, Madras, fixed in FAA, dehydrated in ethanol xylol series and embedded in paraffin wax. Sections were cut 8–10 μm and triple stained with tannic acid-ferric chloride-Heidenhains Iron alum haematoxylin and erythrosin. Leaves and shoot apices were cleared using 5–10% NaOH solution and epidermal peelings were taken using 10% chromic acid.

3. Observations

Tabebuia pentaphylla a member of Bignoniaceae. bears trifoliate to pentafoliate palmately compound leaves with reticulate venation. The terminal leaflet has the longest petiole and the petioles of first pair of leaflets are comparatively shorter, while last pair of lateral leaflets are almost sessile. The phyllotaxy is opposite decusate.

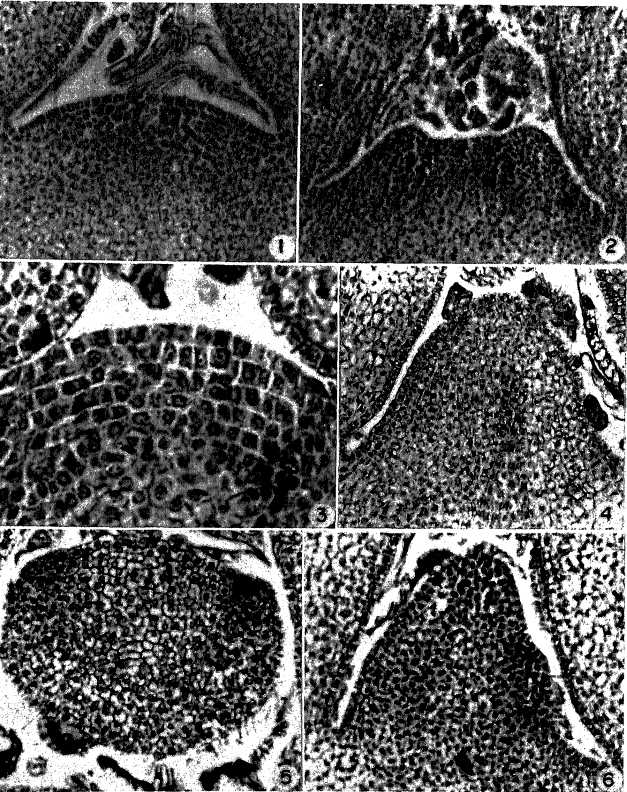
3.1 Apical organisation

The maximal apex is convex measuring 300–325 μm in diameter and 50 μm in height (figure 1). The minimal apex is almost flat measuring 140 μm in diameter (figure 2). Even though two anticlinally dividing outer layers are common, occasionally 3 layers are seen. Periclinal divisions occur in the second layer at the time of leaf and bud initiation. The corpus divides in all planes but may become stratified at times and show more than 4 anticlinally dividing layers at the flanks (figure 3). Regular longitudinal files of cells are produced by repeated transverse divisions at the base of the corpus. There is no clear cut cytohistological zonation.

3.2 Leaf initiation

The first sign of leaf initiation is periclinal divisions in the hypodermal layer at the flank of the shoot apical meristem. This is followed by anticlinal divisions in the adjacent portions of the tunica layer and random division of the subjacent corpus layers to result in the formation of a protuberance (figure 2).

Till the protuberance reaches a height of 50–60 μm , its growth is diffuse by cell division throughout its tissue (figure 4). After this, the primordium begins to thicken dorsiventrally and a conspicuous adaxial meristem arises at the basal region. The adaxial meristem divides by periclinal divisions of the hypodermal layers to produce radial files of cells (figure 5). When the primordium reaches a height of 100 μm , a procambial strand differentiates acropetally in continuation with the procambial strand of the axis. Simultaneously, the wave of tissue maturation commences on the abaxial side at the base and progresses both adaxially and acropetally.



Figures 1-6. *Tabebuia pentaphylla*. 1-2. L.S. of shoot apex at the maximal and minimal phases respectively, $\times 100 \times 215$. 3. L.S. of shoot apex with stratified corpus $\times 585$. 4. T.L.S. of leaf primordium exhibiting diffuse initial growth $\times 285$. 5. T.S. of basal portion of leaf primordium showing adaxial meristem with radial files of cells $\times 215$. 6. T.L.S. of leaf primordium at the time of initiation of first lateral pair of leaflets; section has passed through one leaflet primordium on the right side only $\times 325$.

3.3 Leaflet initiation

The primordia of the first pair of lateral leaflets arise in the leaf primordium when it reaches a height of about $150\text{ }\mu\text{m}$ and before the differentiation of a well defined marginal or laminal plate meristem (figure 6). At about $50\text{ }\mu\text{m}$ below the tip of the primordium, periclinal divisions in the hypodermal layer initiate the primordia (figure 6). The primordia of the second pair of leaflets arise below those of the first when the latter reaches a length of $80\text{--}100\text{ }\mu\text{m}$. The location in which the leaflets are initiated corresponds to the place where the marginal meristem is usually formed in the simple leaf of dicots (figure 7). The terminal portion of the leaf axis becomes the terminal leaflet. Thus, leaflet development is basipetal.

The procambial strands of the midveins of the respective leaflets are initiated prior to their visible outward inception and in fact the inception takes place at the terminal end of a progressing procambial differentiation.

3.4 Lamina initiation

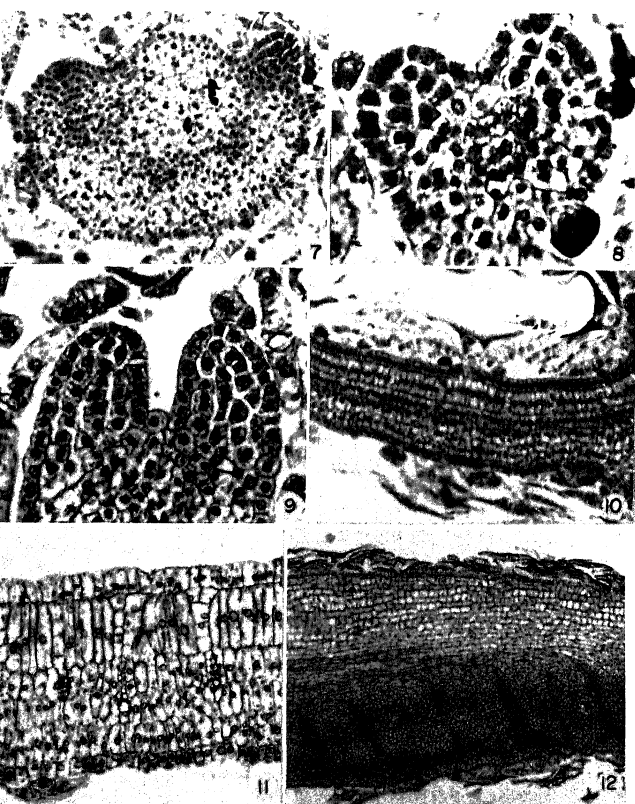
Usually the lamina is first initiated in the terminal leaflet at the fourth plastochron when the leaf primordium reaches a height of $250\text{ }\mu\text{m}$. This is followed by lamina initiation in the first pair of leaflets and lastly in the second pair of leaflets. The marginal meristem that gives rise to the lamina comprises the marginal and submarginal initials and their immediate derivatives. The marginal initials give rise to protodermal initials by anticlinal divisions. The mesophyll of the leaves is produced by the activity of submarginal initials. In the marginal as well as submarginal meristem, it has not been possible to establish a definite demarcation between the initials and their immediate derivatives because of the absence of any morphological or cytological distinction between the two.

The submarginal initial is more or less wedge shaped with two cutting faces (figure 8) and produces an abaxial and an adaxial layer by anticlinal divisions (figure 8). However, the submarginal initial itself may divide anticlinally also (figure 9) and the derivative divide to form the adaxial and abaxial layers. The abaxial layer produces a middle layer by a periclinal division at varying distance from the leaf margin (figure 9). The middle of these 3-layers again undergoes one more periclinal division to organise very early in the ontogeny of the lamina, a 6-layered plate meristem (figure 10). The marginal meristem is active only for a short period until the lamina reaches a length of 25 mm and a width of 2 mm .

Further growth of the lamina takes place by the activity of the 6-layered plate meristem (figure 10) which extends from the midrib to the margin of the leaflet primordium. The cells of the abaxial hypodermis adjacent to the middle layer divide periclinally to form 4-layers of spongy parenchyma. The number of cell layers in a lamina of 3 mm length, still enfolded within the bud is the same as in mature leaf. A mature leaf has a single layer of palisade parenchyma and 4–5 layers of spongy parenchyma (figure 11). The lamina increases in thickness by cell enlargement and by development of intercellular space.

3.5 Vein ontogeny—Major veins

The leaflet is reticulately veined. The venation is comptodromous and characterised by the termination of secondary veins within the margins. The secondary veins are



Figures 7–12. *Tabebuia pentaphylla*. 7. T.S. of leaf primordium showing leaflet initiation at the adaxial margins $\times 240$. 8. T.S. of leaflet primordium with wedge shaped submarginal initials $\times 835$. 9. T.S. of leaflet primordium showing anticlinally dividing submarginal cell (left side) $\times 910$. 10. Six-layered plate meristem in T.S. of expanding leaflet lamina $\times 445$. 11. T.S. of nearly mature leaflet lamina $\times 340$. 12. Paradermal section of expanding leaflet lamina showing diverging procambial strands of the secondary veins $\times 60$.

manner on either side of the midrib and the tertiary veins are irregularly distributed. The midrib region of each leaflet is a composite structure comprising several independent vascular strands, each of which eventually diverges into the lamina as a secondary vein. The course of the vascular strands is very distinct in cleared preparations of the lamina.

The vascularisation of the leaf starts when a procambial strand from the stem deviates from the vascular cylinder and progresses acropetally into the leaf primordium. The first procambial strand develops at the abaxial side of the leaf primordium and progresses acropetally till it reaches the terminal portion. This becomes the precursor of the midvein of the terminal leaflet. Two lateral strands develop on either side of first formed one and enter the first two lateral leaflets as their midveins. Lastly two more strands develop acropetally and lateral to those of the first pair of leaflets and these form the precursor of the midveins of the last pair of leaflets. Thus at a very early stage of ontogeny, 5 independent midvein strands extend into the leaf from the stem. The node is of unilacunar multitrace type.

3.6 *Secondary veins*

Within a leaflet, the primordia of different strands originate basipetally on either half. However, the procambium of secondary strands first originate at the place where the leaflet joins the rachis and develops both basipetally and acropetally. Nevertheless, the differentiation of the vascular elements in the secondary strand is always acropetal at various levels at the base of the rachis. The secondary strands either unite among themselves or with primary strands to form the leaf trace components.

In a developing leaf, the vascular elements of the secondary strands seem to be discontinuous because of the rapid growth produced by the intercalary meristem just below the region where the leaflets join the rachis. However, this discontinuity is eliminated by the subsequent differentiation of vascular elements in the gaps.

The procambial secondaries of the lamina differentiate in an uninterrupted progression from the midvein towards the margin in continuation with the already differentiated secondary strands running parallel to the midvein (figure 12).

3.7 *Veins of higher order*

The tertiary veins divide the intersecondary panel into many bigger areoles. The panel formed by the tertiary veins is further divided by the procambium of quaternary veins into smaller areoles. The procambium of the fifth order veins divide the areoles formed by the quaternary veins into ultimate areoles. The ultimate areoles are not provided with free vein endings.

4. Discussion

As per the tunica corpus concept, all the outer layers that divide anticlinally in the shoot apex, have been designated as tunica. However, Periasamy and Swamy (1964) and Periasamy (1983) have shown that the outermost layer, from which the protoderm and ultimately the epidermis differentiate, cannot be morphologically equivalent to the

shows two or occasionally three additional dividing outer layers which may further increase at times of stratification. Such variability which is due to the behaviour of the corpus alone does not support labelling the layers other than the outermost as tunica in the shoot apex. According to the terminology suggested by Periasamy (1983) the shoot apex of *Tabebuia* is differentiated into a dermogen, hypogen and axiogen.

As in most angiosperms, leaf initiation in *Tabebuia* begins with periclinal division in the hypodermal layer of the shoot apical meristem. The early growth of primordium is rather diffuse, contrary to the apical growth with a single apical cell reported in tobacco (Avery 1933), or by a group of sub apical initials in *Acacia* and *Polyalthia* (Boke 1940; Ramji 1961).

A significant feature of *Tabebuia* is the development of an adaxial meristem at the basal region when the primordium reaches a height of 50–60 μm . The presence and activity of such an adaxial meristem has also been reported in *Acacia* and *Acorus* (Boke 1940; Kaplan 1970a). The adaxial meristem has been variously interpreted. Knoll (1948) and Thielke (1948) interpret the adaxial meristem of the unifacial leaves as the arrested primary apex of the primordium. However, Kaplan (1970a) has shown that in *Acorus* the adaxial meristem is responsible for lamina formation in the unifacial leaves and it is not the arrested primary apex. In *Tabebuia* the adaxial meristem is not so conspicuous as in *Acorus* and contributes to the radial thickening of the primordium.

The previous workers on the ontogeny of the compound leaf (Foster 1936; Denne 1966; Hagemann 1970; Kaplan 1970b; Coleman and Greyson 1976; Merrill 1979) have reported that in both pinnately and palmately compound leaves, the leaflets are formed by fractionation of an initial marginal meristem that would produce a continuous simple lamina. The initiation of the leaflets in *Tabebuia* before the differentiation of a well defined marginal meristem in the leaf primordium, however, does not support this view. Furthermore the leaflet meristem has other fundamental differences from the lamina initiating marginal meristem. The lamina initiating marginal meristem is a characteristically 5-layered plate that undergoes only surface extension whereas the leaflet meristem is more than 5-layered and grows longitudinally as does the leaf primordium. The marginal meristem directly gives rise to the lamina where veins develop subsequently from the middle layer whereas the leaflet meristem first forms the future midrib portion of the leaflet on which the lamina is initiated subsequently by a marginal meristem along its lateral face.

It is more probable that the formation of compound or simple leaf is correlated with the development of the provascular strands of the leaf. In compound leaves, the procambia of the future midribs of the leaflets appear to be formed precociously so that the leaflet primordia arise at the terminal end of a differentiated procambium of the future midrib of the leaflet. In simple leaves, the procambia of the lateral veins are formed from the middle layer of the plate meristem that is derived from the marginal meristem.

As in all plants with imparipinnate leaves, in *Tabebuia* also, the terminal portion of the leaf primordium forms the terminal leaflet. The basipetal leaf initiation in *Tabebuia* seems to be common to all palmately compound leaves.

As the marginal and submarginal initials do not display any cytomorphological difference from their immediate derivatives, their presence and activity is to be presumed by observing occasional divisions in them. It cannot also be determined with certainty whether these initials are permanent or are replaced periodically. The relation

Histogenesis of the lamina commences when it expands by the 5 or 6 layered plate meristem in *Tabebuia* and this appears to be a feature that is general for all the angiosperms irrespective of whether the lamina is compound or simple. During the histogenesis and maturation, the number of layers does not usually increase due to the strictly anticlinal divisions of the plate meristem except at the region of the veins as has been reported in *Xanthium* (Maksymowych and Wochok 1969).

References

- Arber A 1918 The phyllode theory of the monocotyledonous leaf, with special reference to anatomical evidence; *Ann. Bot.* **32** 465–501
- Avery G S Jr 1933 Structure and development of the tobacco leaf; *Am. J. Bot.* **20** 565–592
- Boke N 1940 Histogenesis and morphology of the phyllode in certain species of *Acacia*; *Am. J. Bot.* **27** 73–90
- Coleman W K and Greyson R I 1976 The growth and development of the leaf in tomato (*Lycopersicon esculentum*) II. Leaf ontogeny; *Can. J. Bot.* **54** 2704–2717
- Denne M P 1966 Leaf development in *Trifolium repens*; *Bot. Gaz.* **127** 202–210
- Eames A J 1953 Neglected morphology of the palm leaf; *Phytomorphology* **3** 172–189
- Eames A J 1961 *Morphology of the Angiosperms* (New York: McGraw-Hill)
- Foster A S 1936 Leaf differentiation in angiosperms; *Bot. Rev.* **2** 349–372
- Hagemann W 1970 Studien zur Entwicklungsgeschichte der Angiospermenblätter; *Bot. Jb.* **90** 297–413
- Henslow G 1911 The origin of monocotyledons from dicotyledons through self-adaptation to a moist or aquatic habit; *Ann. Bot.* **25** 717–744
- Kaplan D R 1970a Comparative foliar histogenesis in *Acorus calamus* and its bearing on the phyllode theory of monocotyledonous leaves; *Am. J. Bot.* **57** 331–361
- Kaplan D R 1970b Comparative development and morphological interpretation of “rachis-leaves” in Umbelliferae; in *New Research in Plant Anatomy*, (eds) N K B Robson, D F Cutter and M Gregory *Suppl. I. Bot. J. Linn. Soc. London* **63** 101–125
- Knoll F 1948 Bau, Entwicklung und morphologische Bedeutung unifazialer Vorlauferspitzen an Monokotylenblättern; *Oesterr. Bot. Z.* **95** 163–193
- Maksymowych R and Wochok Z S 1969 Activity of marginal and plate meristems during leaf development of *Xanthium pennsylvanicum*; *Am. J. Bot.* **56** 26–30
- Merrill E K 1979 Comparison of ontogeny of three types of leaf architecture in *Sorbus L*; *Bot. Gaz.* **140** 328–337
- Muruganathan E A 1982 *A comparative study of the ontogeny of non-palm compound leaves in monocotyledons and that of a dicotyledon*, Ph.D. thesis, University of Madras, Madras
- Periasamy K 1965 Morphological and ontogenetic studies in palms II. Growth pattern of the leaves of *Cocos nucifera* and *Borassus flabellifer* after the initiation of plications; *Aust. J. Bot.* **13** 225–234
- Periasamy K 1967 Morphological and ontogenetic studies in palms: V. Early ontogeny and vascular architecture of the leaf of *Rhapis flabelliformis*; *Aust. J. Bot.* **15** 151–159
- Periasamy K 1983 Apical growth in plants; *SBCI News Lett.* **2** 40–52
- Periasamy K and Swamy B G L 1964 Is the microsporangium of angiosperms wall-less? *Curr. Sci.* **33** 735–738
- Ramji M V 1961 Histogenesis of the venation pattern in the leaves of *Polyathia longifolia*; *Proc. Indian Acad. Sci.* **B53** 98–106
- Roth R M 1949 Zur Entwicklungsgeschichte des Blattes, mit besonderer Berücksichtigung von Stipular und Ligularbildungen; *Planta* **37** 299–336
- Sporne K R 1974 *The Morphology of Angiosperms* (London: Hutchinson and Co. Ltd)
- Thielke C 1948 Beiträge zur Entwicklungsgeschichte und zur Physiologie panaschierter Blätter; *Planta* **36** 2–33
- Wilder G J 1976 Structure and development of leaves in *Carludovica palmata* (Cyclanthaceae) with reference to other Cyclanthaceae and Palmae; *Am. J. Bot.* **63** 1237–1256
- Wilder G J 1981 Structure and development of *Cyclanthus bipartitus* with reference to other Cyclanthaceae. II Adult leaf; *Bot. Gaz.* **142** 222–236

RCA₁-mediated agglutination and fluidity of *Mimosa pudica* chloroplast envelope membrane

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Abstract. The thermotropic phase transition temperature in the Chloroplast envelope membrane of *Mimosa pudica* has been measured using a fluorescent probe pyrene, for the first time. RCA₁-induced agglutination does not change the fluidity picture in general but reduces the fluidity uniformly throughout the whole range of temperature (15°–55°C). The turbidimetric assay of this agglutination reveals that this is less in case of membrane than in liposome, for which several explanations are discussed. Also, we have shown that divalent cations, like Ca²⁺ and Mg²⁺ do not affect the lectin-induced agglutination.

Keywords. *Mimosa pudica*; phase transition; chloroplast envelope membrane; RCA₁-induced agglutination.

1. Introduction

Because of its extreme sensitivity to various stimuli, the plant *Mimosa pudica* had been the subject of investigation since as early as 1926 (Bose 1926). We report here our study of the fluidity picture and the thermotropic phase transition within the chloroplast envelope membrane of *M. pudica* by using an excimer-forming fluorescent probe pyrene, a probe quite frequently used in the study of both model and biological membranes (Galla and Sackman 1974; Sengupta *et al* 1976; Flamm *et al* 1982; Nandy *et al* 1983). The landscape of the surfaces of the membrane and the liposome prepared from the polar lipid isolates therefrom, had been surveyed by assaying turbidimetrically the biospecific interaction of RCA₁, a lectin from *Ricinus communis*, with the sugar residues of the membrane glycoprotein and/or glycolipids (Rendi *et al* 1976). The effect of divalent cations Ca²⁺ and Mg²⁺ on this agglutination had been investigated and reversal of agglutination by addition of galactose had been performed. The lectin induced agglutination did not affect the qualitative nature of the membrane phase transition profile, except that there was a general decrease in the fluidity.

2. Materials and methods

RCA₁ was isolated from locally available *R. communis* (Nicolson and Lacorbiere 1973).

Chloroplast envelope membrane was separated from the fresh leaves of *M. pudica* (Poincelot 1973). Lipids were isolated (Bligh and Dyer 1959) and liposome was prepared by sonication of the polar lipid dispersion (Sengupta *et al* 1982) in PBS buffer.

Pyrene was incorporated within the membrane (0.3 mg pyrene per 1 ml of membrane suspension in 50 mM HEPES buffer at pH 7.2, containing 5 mg protein) for

et al 1976). Final concentration of lectin in the assay system was 70 $\mu\text{g}/\text{ml}$ (buffer: HEPES for membrane suspension and PBS for liposome preparation).

In order to study the effect of divalent cations on the RCA_1 -induced agglutination, the assay was carried out (Rendi *et al* 1979) in presence of 2 mM divalent cations Ca^{2+} (as in CaCl_2) and Mg^{2+} (as in MgCl_2). Reversal of agglutination was measured after addition of galactose (10 mM) to the assay mixture.

3. Results and discussion

The ratio of intensity peaks at 485 nm and 390 nm (I'/I) of the pyrene-probed membrane, when illuminated at 320 nm is a measure of the fluidity of the probe's environment (Galla and Sackman 1974) and the change in slope in I'/I versus temperature diagram indicates a phase transition (Flamm *et al* 1982) at that temperature.

In the chloroplast envelope membrane, a distinct phase transition is obtained around 20°C (figure 1A) showing that like most of the other biological membranes, this membrane is also fluid at the ambient temperature. ESR study reports a transition of phase in the chloroplast envelope membrane of other plants in the range 10°–30°C

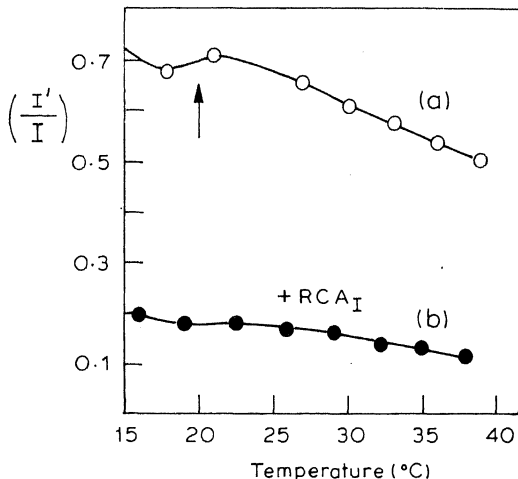


Figure 1. Thermotropic phase transition of chloroplast envelope membrane of *Mimosa pudica*. (a) \circ , normal phase transition, (b) \bullet , in the presence of 70 $\mu\text{g}/\text{ml}$ of RCA_1 . Arrow indicates approximate phase transition temperature.

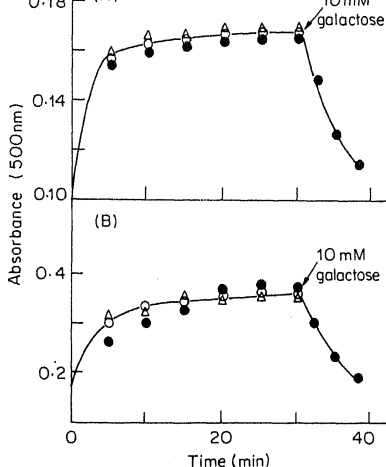


Figure 2. RCA₁-induced agglutination. (A) Chloroplast envelope membrane of *M. pudica*. (B) Liposome prepared from its polar lipid isolate. (O) Without metal ion; (●) with 2 mM MgCl₂; (Δ) with 2 mM CaCl₂.

whereas quite a few other techniques detect a major transition below 0°C (Quinn and Williams 1978). Due to technical limitations, the low temperature transition could not be observed here. In the turbidimetric assay of the interaction of RCA₁ with the membrane and the liposome prepared from its lipid isolates, it is observed that lectin agglutination is more in liposome compared to that in the membrane (81.8% compared to 21.8%, figure 2). This leads us to the conclusion that more receptors are available on the liposome surface than that on the membrane (may also be due to the presence of more lipid molecules). In order to explain this enhanced agglutination, we suggest either one or both of the following possibilities:

(i) The glycolipids and the phosphatidyl inositol contents of the chloroplast envelope membrane of *M. pudica* (Duttachoudhury and Chakrabarti 1980) are the possible lectin receptors. In liposomes the 'cryptic' binding sites of the receptor glycolipids are exposed, whereas these lectin-binding sites are probably shielded by the proteins, specially extrinsic, in membrane preparation (Gordon *et al* 1977).

(ii) In the membrane, proteins interact with endogenous glycosylated receptors forming 'self-neutralised' closed complex (Bowles 1979) which are disrupted in liposomes when proteins are removed from the system.

The study on the effect of divalent cations shows that addition of Ca²⁺ and Mg²⁺ ions does not change the RCA₁-mediated agglutination sufficiently (figure 2). The binding of the lectin in both the membrane and the liposome can be reversed by the

In a separate set of experiments, we have studied the effect of this RCA₁-induced agglutination on the fluidity profile of the envelope membrane. Addition of lectin has not changed the qualitative nature of the phase transition curve but there is a significant decrease in the I'/I value in the experimental range (figure 1B). This can be explained by anticipating proximity of the RCA₁ receptors in the membrane which allows more cross-bridge formation by RCA₁, imparting an over-all rigidity to the system. As a result, I'/I decreases indicating a less fluid environment.

Due to the similarity between the lectin-receptor interaction and antigen-antibody reaction, the observed results are of considerable biological significance.

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References

- Bligh E G and Dyer W J 1959 A rapid method of total lipid extraction and purification; *Can. J. Biochem. Physiol.* **37** 911-917
- Bose J C 1926 in *The Nervous Mechanism of Plants*, (London: Longmans, Green and Co. Ltd) p. 44, 89, 105, 176
- Bowles D J 1979 Lectin as membrane components: implications of lectin-receptor interaction; *FEBS Lett.* **102** 1-3
- Duttachoudhury M and Chakrabarti P 1980 Cellular and chloroplast lipid composition of the leaves of *Mimosa pudica*; *Phytochemistry* **19** 519-523
- Flamm M, Okubo T, Turro N and Schachter D 1982 Pressure dependence of pyrene excimer fluorescence in human erythrocyte membranes; *Biochim. Biophys. Acta* **687** 101-104
- Galla H J and Sackman E 1974 Lateral diffusion in the hydrophobic region of membranes: use of pyrene excimers as optical probes; *Biochim. Biophys. Acta* **339** 103-115
- Gordon J A, Stahelin L A and Kuettner C A 1977 Lectin-mediated agglutination of erythrocyte ghost membranes following depletion of membrane protein and intermembrane particle; *Exp. Cell. Res.* **110** 439-448
- Nandy P, Duttachoudhury M and Chakrabarti P 1983 Effect of sealing on the incorporation of pyrene in goat erythrocyte ghosts - a fluorescence study; *J. Biosci.* **5** 163-166
- Nicolson G L and Lacorbiere M 1973 Cell contact-dependent increase in membrane D-galactopyranosyl-like residues on normal, but not virus or spontaneously-transformed murine fibroblasts; *Proc. Natl. Acad. Sci.* **70** 1672-1676
- Poincelot R P 1973 Isolation and lipid composition of spinach chloroplast envelope membranes; *Arch. Biochem. Biophys.* **159** 134-142
- Quinn P J and Williams W P 1978 Plant lipids and their role in membrane function; *Prog. Biophys. Mol. Biol.* **34** 109-173
- Rendi R, Kuettner C A and Gordon J A 1976 Agglutination by lectins of liposomes prepared from total lipids of erythrocytes; *Biochim. Biophys. Res. Commun.* **72** 1071-1076
- Rendi R, Vatter A E and Gordon J A 1979 Divalent cation enhancement of the agglutinability by soybean lectin of liposomes prepared from total lipid of erythrocytes and of erythrocyte membranes; *Biochim. Biophys. Acta* **550** 318-327
- Sengupta P, Sackman E, Kuhnle W and Scholz H 1976 An optical study of the exchange kinetics of membrane bound molecules; *Biochim. Biophys. Acta* **436** 869-878
- Sengupta P, Duttachoudhury M, Biswas S K and Chakrabarti P 1982 Studies on phase transition of some erythrocyte ghosts; *Indian J. Exp. Biol.* **20** 513-516

Variation in germination characteristics of some seed origins of *Pinus wallichiana* A B Jacks from the western Himalaya

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Abstract. Seeds of kail collected from 10 localities in the western Himalaya were germinated at 4 temperatures viz 20°, 28°, 34° and 40°C after storage in dry cold conditions in freezer. The seed lots representing different localities differed in the capacity and rate of germination. At lower temperature of incubation (20°C) most of the seed lots exhibited varying degree of dormancy. This dormancy could be revoked by chilling imbibed seeds for 2 weeks at 3–5°C. At higher temperatures of incubation the seed lots did not exhibit dormancy, although the optimum conditions for germination were provided by a combination of prechilling and higher temperatures of incubation (28° and 34°C). Treatments such as prolonging the stratification period up to 38 days, soaking in GA 100 mg/litre, with or without prechilling, and in 1% H₂O₂, did not have any significant effect on germination. The study revealed seed source variation in the degree of dormancy at low temperature of incubation.

Keywords. *Pinus wallichiana*; kail; prechilling; dormancy; seed source.

1. Introduction

Pinus wallichiana A B Jacks (Syn *Pinus griffithii* McClell, Kail or blue pine) is the most important coniferous timber tree of the Himalaya next to deodar (Gamble 1922). The species, except for few gaps is widely distributed all along the entire length of the Himalaya (see Critchfield and Little 1966, Pp. 7 and 43) and has an altitudinal range from 1219–3657 meters, which is greater than any other Himalayan conifer (Troup 1921). The species therefore show much geographic variability. Dogra (1972) recognized 7 altitudinal provenance types of Kail, 4 adapted to the outer moist and inner dry North-West Himalaya and 3 to the outer wet, middle moist and inner dry Eastern Himalaya. In North-Western Himalaya, i.e., Jammu and Kashmir, Himachal Pradesh and Uttar Pradesh, Kail grows in two district zones of a cross-section of the Himalaya, the moist southern outer zone and, the dry northern inner zone behind the main Himalayan ranges which is beyond the reach of the monsoon rainfall. In 4 broad provenance types recognized in North-Western region two are moist low level Kail (1200–1980 meters) and moist upper level Kail (1980–3050 meters) whereas in the inner outer non-monsoon zone, the two are dry low level (1980–3200 meters) and dry or arid high level types (3200–4330 meters). The seed origins discussed in this account belong to outer monsoon zone provenance only.

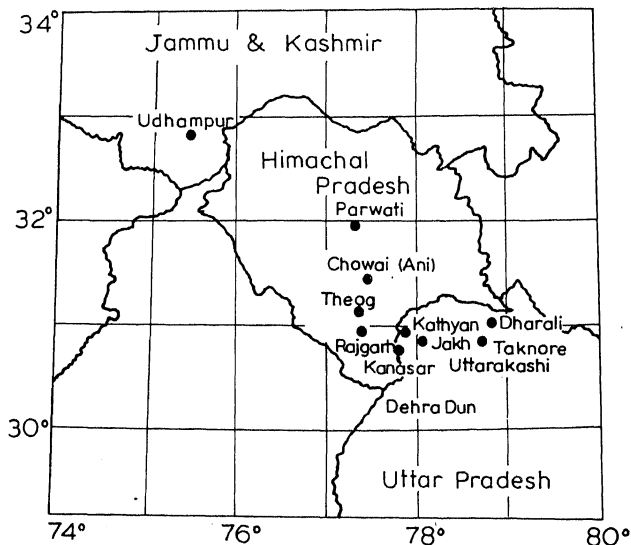
On account of wide adaptation of Kail, different temperature optima for seed germination correlated with the habitat, are expected to occur within the species. Variation in temperature and light requirements for seed germination within or between species has been reported in most Himalayan species (Thapliyal and Gupta 1980; Semwal and Purohit 1980). Allen (1960) studied the germination behaviour of coniferous seed with reference to stratification period and incubation temperatures and

suggested the use of physiological responses of seed in the mapping of the regions of provenances. The present investigation was taken up with the object to gain an insight into the nature of variation in the physiology of seed germination and explore its possible usefulness in differentiating and further narrowing down the broad provenance types described by Dogra (1972).

2. Materials and method

Seeds were collected from 10 places in Jammu, Himachal Pradesh and Uttar Pradesh in the year 1979. As indicated in figure 1 seed origins studied in this paper represent latitudinal and longitudinal profile within a zone of more or less similar monsoon rainfall and altitude with the exception of two places belonging to inner valleys of the river Ganga (Dharali) and Parwati which are subject to weaker monsoon. Cones were collected from 5–10 different, widely separated trees and bulked together. All cone collection, seed extraction and storage procedure was kept uniform for all the seed origins.

A few days exposure to sun followed by shaking and manual twisting of cone facilitated total seed recovery. Seed weight was determined using 8 replications of 100 seed each. Moisture content on duplicate 50 seed sample was determined by oven dry



method at 105°C for 16 hr and expressed on fresh weight basis. Before the commencement of germination test, seeds were stored in sealed polythene bags at 10°C for 14 months.

Seeds from each lot were germinated at constant temperatures, viz 20°, 28°, 34° and 40°C (henceforth referred to as treatments A₁–A₄). Another set was germinated at similar temperatures but after chilling the imbibed seeds for two weeks (treatments B₁–B₄). Treatments A and B were planned initially but owing to high percentage of fresh ungerminated seed in some lots further treatments, before incubating the seed at 28°C, were envisaged with the object to obtain better germination. These additional treatments were as follows:

- (i) Treatment C—Soaking the seed in gibberellic acid (GA) (100 mg/litre) at 28°C for 48 hr.
- (ii) Treatment D—Soaking the seed in Hydrogen Peroxide (H₂O₂; 1 % v/v) at 28°C for 48 hr.
- (iii) Treatment E—Seed treated with GA as above and then chilled for 15 days.
- (iv) Treatment F—Seed chilled for 38 days.

Seeds were chilled by soaking in water at 3–5°C for 24 hrs in a refrigerator. After the removal of excess water the wet seeds were packed in open polythene bags to which ice cold water was added as and when necessary. Germination test was conducted on randomly drawn 4 replications of 100 or 50 seed samples from the ungraded lot and placed between moist paper towels, rolled and kept vertically in bon incubators. A seed was considered germinated when radicle attained the length of about 1 cm. Ungerminated seeds were dissected to find out the percentage of empty seeds in each lots as mentioned in table 2. The rate of germination was calculated and expressed as germination value (GV) according to the following equation (Czabator 1962):

$$GV = PV \times MDG$$

Where, gv is a product of pv, the peak value, which is the highest value of the cumulative germination percentage divided by the number of days since the start of the experiment and MDG is the mean daily germination.

An analysis of variance of $\sin \sqrt{P}$ transformed values of germination as well as gv data was done to evaluate statistically significance of differences in germination due to treatments and interaction with seed origins. Germination data in respect of lot H was not available for some of the treatments and therefore was not included for calculating the cumulative means for treatment, as well as, for the analysis of variance. The results of the statistical analysis have been presented in tables 2–5 alongwith mean germination percentage and gv.

3. Results

A wide range of variation in germination requirement of Kail seed with or without pretreatment is apparent. Both the temperature treatments as well as seed lots accounted for this variation.

3.1 Treatments

Under treatment A the mean germination percentage for all lots was 25.5% and ranged

Seed lot	State	Location	Longitude (°N)	Latitude (°E)	Altitude (m)
A.	Jammu and Kashmir	Charian 82/Dudu Comp. Dudu Range, Udhampur Division	75°-35'	32°-50'	2399
B.	Himachal Pradesh	Parwati Range, Kulu Forest Division	77°-19'	31°-57'	2766
C.	" "	Ani Range, Chowai Comp., Seraj Forest Division	77°-27'	31°-28'	2000
D.	" "	Khatmail Block, Theog Range, Simla Forest Division	77°-19'	31°-8'	1951
E.	" "	Haban Range, Rajgarh Forest Division	77°-22'	30°-58'	2333
F.	Uttar Pradesh	Compartment No. 8 Kanasar Range, Chakrata Forest Division	77°-48'	30°-48'	1800
G.	" "	Kathyan Range, Chakrata Forest Division	77°-53'	30°-57'	2100
H.	Uttar Pradesh	Dharali, Compartment No. 3, Taknora Range, Uttarkashi Forest Division	78°-48'	31°-5'	2666-40
I.	" "	Bhukki, Taknora Range, Uttarkashi Forest Division	78°-42'	30°-53'	2166-45
J.	" "	11-C/Jakh Compt. Purola Range, Yamuna Forest Division	78°-4'	30°-52'	2134

higher temperatures, i.e. treatments A_2 and A_3 the germination percentage increased two to several fold depending upon the lots. Treatment A_4 resulted in decline in germination percentage but the latter was still higher than under treatment A . Following prechilling at 3-5°C for 15 days, germination in all lots increased under treatments B_1 and B_2 but decreased sharply at higher temperatures, i.e. treatments B_3 and B_4 . Bar diagram plotted for treatments revealed that, in general, the treatment B_2 resulted in best germination followed by B_1 , A_2 and A_3 . The differences between these treatments were however, not significant. Treatments A_4 , A_1 , B_4 and B_3 in that order proved to be the worst. GV was highest under treatment B_2 and A_2 followed by B_1 , A_3 and A_4 . Treatments A_1 proved to be the worst.

Within the lots, the germination percentage varied from 23.19% in lot I to 86.11% in lot H. Lots E, H and I were notable exceptions in which germination percentage was

Table 2. Mean germination percentage of 10 lots of kail seed influenced by incubation temperatures and chilling

Seed lot	Percentage of filled seed	Treatments								Cumulative means for lots
		Unchilled				Prechilled				
		A ₁ (20°C)	A ₂ (28°C)	A ₃ (34°C)	A ₄ (40°C)	B ₁ (20°C)	B ₂ (28°C)	B ₃ (34°C)	B ₄ (40°C)	
A	73.88 ± 5.08	7.25	56.00	60.00	40.25	73.00	77.50	4.00	15.50	41.94 ^c
B	79.27 ± 2.56	42.50	82.50	82.00	57.75	72.00	80.00	7.50	27.50	56.47 ^a
C	76.50 ± 1.17	35.50	61.50	62.75	44.25	77.50	74.75	5.50	5.00	45.84 ^b
D	85.00 ± 3.30	9.75	71.75	63.75	38.00	81.50	81.50	1.00	9.50	43.97 ^c
E	80.06 ± 3.75	56.75	68.00	66.75	59.25	64.50	62.75	17.50	47.50	55.38 ^a
F	87.56 ± 3.88	15.25	72.25	70.00	55.00	81.00	74.75	6.00	20.50	49.34 ^b
G	82.43 ± 2.75	1.50	72.75	72.75	51.00	76.00	86.00	1.00	32.00	49.12 ^b
H	90.87 ± 2.59	88.75	91.50	85.75	82.75	90.50	91.50	—	—	88.79
I	81.06 ± 1.49	58.75	76.25	65.00	66.50	78.50	79.00	4.00	17.00	55.63 ^a
J	71.43 ± 4.72	7.50	31.75	41.75	23.75	34.00	38.75	9.50	5.00	23.19 ^d
Cumulative means for treatments		25.53 ^c	65.86 ^a	64.97 ^a	48.42 ^b	70.89 ^a	72.78 ^a	6.22 ^d	19.94 ^c	

Cumulative means for seed lots and treatment followed by the same suffix letter are not significantly different ($P < 0.01$) according to Scheffe's method.

Table 3. Mean germination percentages of 10 lots of kail seed influenced by pre-germination treatments.

Seed lot	Treatments				Cumulative means for the lots
	GA(100 mg/litre) (C)	H ₂ O ₂ (1 % V/V) (D)	GA (100 mg/litre) + prechilled for 15 days (E)	Prechilled for 38 days (F)	
A	43.00	62.50	67.50	69.00	60.50 ^b
B	56.50	71.00	77.00	76.50	70.25 ^a
C	39.50	44.00	65.50	71.25	55.05 ^c
D	61.00	54.00	88.00	82.75	71.44 ^a
E	24.00	46.00	63.50	67.00	50.13 ^c
F	62.50	68.00	82.00	77.25	72.44 ^a
G	47.50	63.00	73.00	76.75	65.06 ^b
H	—	66.50	89.50	88.25	61.06
I	10.50	66.50	81.00	77.00	58.75 ^b
J	13.50	23.00	27.00	42.25	26.44 ^d
Cumulative means for treatments	39.78 ^c	56.45 ^b	71.40 ^a	72.80 ^a	

Cumulative means for seed lots and treatment followed by the same suffix letter are not significantly different ($P < 0.01$) according to Scheffe's method.

Table 4. The mean GV of 10 lots of kail seed influenced by incubation temperatures and chilling

Seed lots	Treatments							Cumulative means for the lots
	Unchilled				Prechilled			
	A ₁	A ₂	A ₃	A ₄	B ₁	B ₂	B ₄	
A	0.09	12.31	4.71	1.76	11.70	12.10	0.52	6.17 ^d
B	2.75	29.52	12.19	3.80	18.96	38.26	1.56	15.40 ^a
C	1.92	14.60	4.81	2.14	11.98	15.78	0.14	7.27 ^c
D	0.04	18.26	4.99	1.45	10.54	19.90	0.22	7.91 ^c
E	4.63	17.57	8.18	6.91	8.06	12.47	4.72	9.07 ^b
F	0.27	20.09	6.58	3.36	11.26	13.17	0.88	7.77 ^c
G	0.00	11.65	6.70	2.59	7.34	19.12	2.06	7.06 ^c
H	14.69	37.57	12.43	9.96	32.30	27.72	—	22.45
I	4.26	21.70	5.66	4.58	16.06	18.60	7.50	10.23 ^b
J	0.07	2.09	2.01	0.69	1.54	2.09	0.74	1.22 ^e
Cumulative means for treatments	1.56 ^d	16.42 ^a	6.20 ^c	3.03 ^c	10.83 ^b	16.83 ^c	2.04 ^d	

Seed lot	Treatments				
	GA(100 mg/litre) (C)	H ₂ O ₂ (1 % V/V) (D)	GA (100 mg/litre) +prechilled for 15 days (E)	Prechilled for 38 days (F)	Cumulative means for the lots
A	3.08	4.41	15.61	9.49	8.15 ^c
B	5.49	8.20	33.21	34.55	20.36 ^a
C	2.90	1.82	12.01	27.12	10.96 ^b
D	5.63	3.80	22.13	52.58	21.04 ^a
E	0.83	2.67	14.88	20.22	9.55 ^b
F	3.78	4.33	14.82	11.50	8.61 ^c
G	5.06	5.78	18.46	11.55	10.21 ^b
H	—	—	18.96	21.50	20.13
I	0.30	5.61	17.11	14.62	9.41 ^b
J	0.23	0.63	3.64	1.12	1.42 ^d
Cumulative means for treatments	3.03 ^b	4.14 ^b	16.87 ^a	20.31 ^a	

Cumulative means for seed lots and treatment followed by the same suffix letter are not significantly different ($P < 0.01$) according to Scheffe's method.

uniform under treatments A₁–A₄, B₁ and B₂. A far greater amount of variation was discernible in the rate of germination (Gv) within seed lots and treatment. In lots E, H and I for example, in which although there was no significant differences in total final germination yet the germination value differed significantly (table 3). The overall germination value ranked highest among treatments A₂ and B₂, and in these treatments the differences were not significant. The combination of prechilling at 3°–5°C and higher temperatures of incubation appeared to have pronounced inhibitory effect on germination. It is evident from the fact that without prechilling the germination at 40°C was on an average 41.83% but fell down to 7.9% after incubation at the same temperature following prechilling for 15 days. The very low germination and almost zero Gv at 34°C of prechilled seed was on account of anaerobic conditions created inside the incubator due to fault in air circulation system detected later, and hence was excluded from all the statistical computations.

3.2 Seed origins

The seed lots used in the study belong to a small and narrow altitudinal range (1800–2766 meters) within the monsoon zone of 3 states viz. Jammu, Himachal Pradesh and Uttar Pradesh, thus rendering the sampling fairly uniform. The latitudinal (30°48' to 32°57') and longitudinal (75°35' to 78°48') range is however sufficiently wide to show significant genetic differences. Of the 10 lots used in this study, 4 lots viz., A, B, E and H, represent specific geographic sources as defined by the latitude and longitude of their origin in the western Himalaya whereas the remaining lots represent minor elevational changes within these sources. It is interesting, the manner in which seeds of the 4 origins respond to incubation temperatures and stratification. The germination percent of

Comparisons of response of chilling within lots however indicated that the level of significance between control and treated seeds was highest in lot A and declined gradually through lots B and E and became non-significant in lot H (figure 2).

The additional treatments F and E did not result in further improvement in germination percentage as well as in germination value (table 3). These treatments were, more or less, comparable to treatments A₂, A₃, B₁ and B₂ and fell under the same bar. Treatments D and C proved worse in descending order.

4. Discussion

Germination studies on kail, under laboratory conditions have received attention from several workers. International Seed Testing Association (1976) have recommended temperatures alternating between 20°C for 16 hr and 30°C with light for 8 hr during a 24 hr regime as the optimum condition for routine germination test. On the other hand AOSA (1965) recommend same as ISTA with 8 hr daily light period and an incubation period of 18 days. Rafn (1915) on the basis of germination studies on 12 samples at lower temperatures, i.e. 24.5°C/21.25°C and total darkness, reported 64 % germination

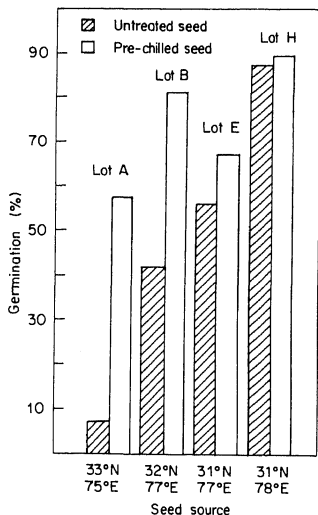


Figure 2. Variation in the prechilling requirement of kail seed from 4 different sources of the Western Himalaya.

temperatures ranging from 20 °C to as high as 34 °C and 40 °C and, as revealed in the present investigation, this capacity appears to be more characteristics of the seed sources belonging to the weak monsoon zones, e.g., the Dharali and Parwati (figure 3). More evidence on this has been obtained in, as yet inconclusive, investigations comparing germination behaviour of seed from the dry or arid non-monsoon zones with those of the high rainfall zone. Normally, in the region of kail, the absolute minimum and maximum shade temperatures range from -17° – 38° C (Troup 1921). In an arid non-monsoon zone, where vegetation cover is scanty, the diurnal temperature variations may be even more and, on exposed sites, during the day, has been reported to increase with an increase in altitude from 35° – 73° C (Mani 1978). Troup (1921) mentioned that kail seed, following dispersal, lies on ground throughout the winter and during hot season and commence to germinate only in the rainy season. He recognized late germination of kail seed and the beneficial effect of the snow cover but related it to the protection it offered from predators. According to Heit (1968) kail seed exhibit little or no dormancy but has been reported to relapse into one consequent upon storage for prolonged periods.

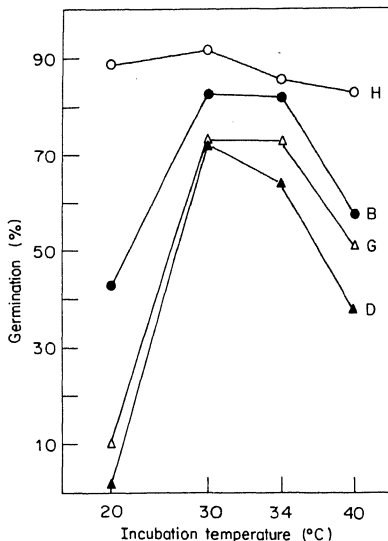


Figure 3. Germination characteristics of *P. wallichiana* seed from weak monsoon zone (lots B and H) and heavy monsoon zone (lots D and G).

-20°C (Siddiqui and Parvez 1981) has been suggested to overcome this dormancy. In the present investigation it was interesting to note, however, that the dormancy was manifest only at low temperatures of incubation and could be revoked either by prechilling or by higher temperatures of incubation i.e., 28°C and 34°C or by a combination of both the prechilling as well as incubation at 28°C. The induction of dormancy during dry cold storage has been observed in conifer (Vanesse 1974) as well as broad-leaves species (Wang and Haddon 1978). The dormancy in the former case could be overcome by 8 weeks stratification. Vanesse (1974) studied the nature of seed dormancy induced during dry cold storage and observed that there was a gradual reduction in dormancy during germination at optimum temperatures and that the process was strongly influenced by the incubation temperatures. He suggested that the degree of dormancy is a germination characteristic peculiar to every seed lot and the way in which dormancy changes after several years storage under dry conditions is different for each lot.

The pattern of germination in 4 seed sources indicate a higher percentage of dormant seed necessitating a greater requirement of stratification in seeds from the North-Western Himalaya. The degree of dormancy gradually declines from North-West to South-East and the source from Dharali in Uttarkashi District of Uttar Pradesh, representing the eastern most origin in the present investigation, exhibits almost no dormancy.

The above observation may, at best, be considered a preliminary one since the seed lots used in this study belong only to a small area in the Western Himalaya. Stratification requirement of seed from different populations have been studied in several conifer species (Haasis and Thrupp 1931; Stearn and Olson 1958; Olson *et al* 1959; Allen 1960; Mergen 1963; Fowler and Dwight 1964). In all these studies the stratification requirement, for uniform and a higher percentage or rate of germination, has been related with the latitude and/or mean annual temperature or with the longitude (Popov 1982) of the seed source. In the light of the uniformity of elevation and rainfall the sampling area of present investigation is simply a geographic unit conforming more or less to one of the provenance types of Dogra (1972) i.e., the low level monsoon or weak monsoon zone kail of the western Himalaya. It is therefore significant if such physiological parameters as used here can help in sub-dividing the zone of blue pine over a latitudinal and longitudinal cline and according to rainfall pattern.

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References

- Allen G S 1960 Factors affecting the viability and germination behaviour of coniferous seed, IV, stratification period and incubation temperature, *Pseudotsuga menziesii* (Mirb) Franco; *For. Chron.* 36 18-29

- Association of Official Seed Analysts 1965 Rules for testing seeds; *Proc. Assoc. Off. Seed Anal.* **54** 1-112
- Czabator F J 1962 Germination value: an index combining speed and completeness of pine seed germination; *For. Sci.* **8** 366-396
- Critchfield W B and Little E L 1966 *Geographic distribution of the pines of the world*, US Department of Agriculture, Forest Service, Miscellaneous Publication, **991** 1-97
- Dent T V 1947 Seed storage with particular reference to the storage of seed of Indian Forest Plants; *Indian For. Rec. (N.S.) Silv.* **7** 1-134
- Dogra P D 1972 Intrinsic qualities of growth and adaptation potential of *Pinus wallichiana*; in *Biology of Rust Resistance in Forest Trees*, (eds) R T Bingham, R J Hoff and G I McDonald (Washington: USDA Forestry Service Misc. Publ. 1221), pp 163-178
- Fowler D P and Dwyer T W 1964 Provenance differences in the stratification requirements of white pine; *Can. J. Bot.* **42** 669-675
- Gamble J S 1922 *A manual of Indian Timbers* (London: Sampson Low, Marston and Company) pp 7-760
- Haasis F W and Thrupp A C 1931 Temperature relations of Lodge pole pine seed; *Ecology* **12** 728-744
- Heit C S 1968 Propagation from seed; part 12 growing choice, less common pines; *Am. Nurseryman* **127** 112-120
- International Seed Testing Association 1976 International Rules for Seed Testing; *Proc. Int. Seed Test. Assoc.* **4** 3-177
- Mani M S 1978 Ecology and Phytogeography of high altitude plants of North-West Himalaya; (New Delhi: Oxford and IBM) pp 1-205
- Mergen F 1963 Ecotype variation in *Pinus strobus* L.; *Ecology* **44** 716-727
- Olson J S, Stearns F and Nienstaedt H 1959 Eastern hemlock seeds and seedlings, response to photoperiod and temperature; *Conn. Agric. Exp. St. New Haven Bull.* **620** 1-70
- Popov P P 1982 Influence of ecological conditions on the germination of *Picea obovata* seeds; *Lesn. Khoz.* **2** 19-22
- Rafn J 1915 *The testing of Forest seeds during 25 years 1887-1912*, (Copenhagen: Langkjaers Bogtrykkeri) pp 1-91 (Printed for private circulation) cited from: *Pinus L. Pine*, by Krugman S L and Jenkinson J L in *Seeds of the woody plants in the United States*, Forest Service USDA Handbook No. 450, pp 598-638
- Semwal J K and Purohit A N 1980 Germination of Himalayan Alpine and Temperate *Potentilla*; *Proc. Indian Acad. Sci. (Plant Sci.)* **89** 61-65
- Siddiqui K M and Parvez M 1981 Seed storage and germination studies in blue pine; *Pak. J. For.* **31** 51-60
- Stearns F and Olson J 1958 Interactions of photoperiod and temperature affecting seed germination in *Tsuga canadensis*; *Am. J. Bot.* **45** 53-58
- Thapliyal R C and Gupta B N 1980 Effect of Seed Source and Stratification on the germination of deodar seed; *Seed Sci. Technol.* **8** 145-150
- Troup R S 1921 *The Silviculture of Indian Trees* (Oxford: Clarendon Press) vol. 3, 785-1195
- Vanessse R 1974 Changes in the degree of dormancy of Douglas Fir seeds; *Bull. Soc. R. For. Belg.* **81** 149-158
- Wang B S P and Haddon B D 1978 Germination of red maple seed; *Seed Sci. Technol.* **6** 785-790

Role of endogenous phytohormones and some macromolecules in regulation of sex differentiation in flowering plants

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Abstract. Endogenous levels of phytohormones like auxin, gibberellin, cytokinin, abscisic acid and ethylene appear to determine the fate of flower sex primordia. Higher levels of auxins, cytokinins and ethylene have been found to be correlated with female sex expression whereas a greater amount of gibberellins favours the differentiation of male sex organs. It appears that the phenotypic expression of flower sex is controlled by a balance between male-promoting and female-promoting hormones. Variations occurring with regard to amino acids, proteins and enzymes/isozymes during the reproductive phase may also have a prominent role in determining the nature of the flower sex.

Keywords. Phytohormones; macromolecular markers; sex expression.

1. Introduction

Initiation of flowering is one of the very important morphogenetic events in the life of a plant. Much work has been done to discern the factors responsible for initiation of flowering and several theories have been postulated concerning its mechanism (Lang 1952; Chailakhyan 1968; Thornley 1972; Charles-Edwards *et al* 1979). However, information regarding the factors of flower development and sex organ differentiation is insufficient. Exogenously applied phytohormones are known to change the sex of plants (Frankel and Galun 1977; Mohan Ram 1980). Chailakhyan and Khryanin (1979) have demonstrated that during transition from vegetative to reproductive phase, changes in the plant metabolism occur as a result of which certain new and specific macromolecules appear, disappear, or their levels change significantly. These molecules probably constitute molecular markers or probes of differentiation (Champault *et al* 1981). It is not necessary to examine many of these molecules but only the selected ones which are specific to different sexes.

In this review, the possible correlation amongst endogenous levels of phytohormones and some compounds like amino acids, proteins and enzymes/isozymes in relation to flower sex differentiation has been examined.

2. Role of endogenous hormones

2.1 Auxins

Relatively high levels of endogenous auxins are correlated with female sex expression in most of the plants studied. Female plants/flowers of various species show greater

amounts of endogenous auxin than the male plants/flowers (Frankel and Galun 1977).

Galun *et al* (1965) demonstrated that the quantity of auxin in hermaphrodite and andromonoecious cucumber plants, differed significantly, the hermaphrodite ones being richer.

2.2 Gibberellins

Among the 5 groups of plant hormones higher levels of endogenous gibberellins have been detected in the male than in the female plants (Vince-Prue 1975). Gibberellin has been found to be a major factor for enhancing male flower formation in cucumber—the most studied plant regarding the role of hormones on sex expression and a correlation between higher endogenous gibberellin level and male sex expression has been established (Frankel and Galun 1977).

Atsmon *et al* (1968) and Hayashi *et al* (1971) reported considerably higher levels of gibberellin-like substances in a monoecious line of cucumber (*Cucumis sativus* L.) than that in an isogenic gynoeceious line. Greater gibberellin activity has also been recorded in leaves of male plants of hemp and spinach than in female ones (Chailakhyan and Khryanin 1979). However, Leshem and Ophir (1977) reported the presence of higher endogenous level of a gibberellin-like substance in female individuals of two dioecious species, viz. *Ceratonia siliqua* and *Phoenix dactylifera*. Recently, Ghosh and Basu (1983) have detected a high level of endogenous gibberellin-like substance in shoot tips of *Momordica charantia*, a monoecious cucumber, at the time when the plant had a minimum ratio of male to female flowers.

2.3 Cytokinins

Cytokinins have also been found in greater quantities in female plants than in male plants of various species studied. Zeatin was detected only in female flowers of *Mercurialis annua*, whereas its precursor nucleotides were observed in male plants also (Dauphin-Guerin *et al* 1980). Champault *et al* (1981) recorded the feminization of genetically male plants and postulated a hypothesis that female plants contain a larger pool of endogenous cytokinins than male ones.

Chailakhyan and Khryanin (1979) noted that in intact soil-grown plants of *Cannabis sativa* and *Spinacia oleracea*, cytokinin activity was greater in leaves as well as in primary root of female than in those of male plants. These authors recorded lower cytokinin activity in the leaves of derooted male plants of hemp and spinach and concluded that higher levels of natural gibberellins and low levels of cytokinins in the leaves of derooted plants were the major causes of the shift of sex expression in the male direction. Moreover, promotion of development of already differentiated pistils of *Cleome iberidella* grown *in vitro* by zeatin and benzylamino purine was shown by De Jong and Bruinsma (1974).

2.4 Absciscic acid

Higher levels of absciscic acid were recorded in the shoot tips of gynoeceious plants of cucumber than in those of monoecious plants (Rudich and Halevy 1974; Friedlander *et*

al 1977). These investigators concluded that higher content of abscisic acid could be correlated with female flower differentiation and lower abscisic acid content with male flower differentiation.

2.5 Ethylene

It has been demonstrated that comparatively elevated levels of endogenous ethylene favour femaleness in most plants. In *C. sativus* and *C. melo* gynoeccious plants produce more ethylene than their monoecious counterparts. Andromonoecious plants were reported to evolve low amounts of ethylene as compared to monoecious plants and hermaphrodite flowers (Rudich *et al* 1976). The role of endogenous ethylene in femaleness was established in cucurbits by Byers *et al* (1972). They removed ethylene from female plants by ventilation and exposed them to ethylene inhibitors such as CO₂ and benzothiadiazole and observed the induction of hermaphrodite flowers. Gregg (1982) studied the light-enhanced ethylene content in several species of *Cynoches* and *Catasetum* and reported that higher levels of ethylene were emitted by developing female flowers in plants grown in shade, whereas low levels of ethylene were evolved from male inflorescences grown in sunlight. Adams and Yang (1979) have demonstrated 1-aminocyclopropane-1-carboxylic acid (ACC) as the precursor of ethylene biosynthesis and the former has been shown to play its role during certain ethylene mediated activities such as fruit ripening (Hoffman and Yang 1980), senescence (Bufler *et al* 1980) and as actual factor for inducing femaleness in *Cucurbita pepo* (Hume and Lovell 1982).

Recently, it was also demonstrated that natural as well as ethephon-induced female flowers of dioecious *Cannabis sativa* evolve significantly higher amount of ethylene than the male flowers, specially at the early stages of development (Sriram and Mohan Ram 1984). These findings strengthen the concept that higher ethylene content is associated with female sex expression in plants.

3. Role of amino acids

Being a main form of soluble nitrogen within cells and playing a vital role in cell biosynthesis, amino acids are extensively studied molecules (Fowden 1973; Mifflin and Lea 1976; Umberger 1978). Nevertheless, reports on variation in the quantity/kinds of amino acids during transition from vegetative to reproductive stage or differences in male and female flowers are scanty.

During our studies on *Ricinus communis* by paper chromatography, we have shown that arginine appears only in male, leucine only in female and alanine, hydroxyproline and valine in flowers of both sexes but not in vegetative tissues (Jaiswal and Kumar 1980a). At the same time glutamic acid and serine, present in vegetative parts, disappear during the onset of flowering. Further, certain amino acids show differential presence or absence in vegetative and/or reproductive parts or in male and/or female flowers at a particular stage of development (Jaiswal and Kumar 1978; Kumar 1982). During transition from vegetative to reproductive phase, leucine and lysine appear in male flower primordia and lysine disappears in female flower primordia of *Morus nigra*. Higher levels of female flower primordia of *Grewia argentea* and *Thaunus* and

tissues but disappears in female flower primordia. Apart from these, certain other amino acids also showed variations with particular stage of flower development (Kumar 1982).

4. Role of proteins

Earlier reports have shown differences in the protein profiles of vegetative and reproductive structures in a few plants, viz. tulip (Barber and Steward 1968), *Mercurialis annua* (Kahlem 1970; Kahlem *et al* 1973) and *C. papaya* (Jindal and Singh 1976). During flowering, alteration in the antigenic proteins in apical buds along with the disappearance of one protein species and increase in the concentrations of two others were reported in *Sinapis alba* (Pierard *et al* 1977).

The differences in the protein profiles of *M. nigra*, *C. papaya*, *C. indica* and *R. communis* were investigated and it was observed that male (as in *M. nigra* and *C. papaya*) or female (as in *C. indica*) flower buds had specific proteins during the reproductive phase that were not recorded at the vegetative stage. At the same time, certain protein species present in vegetative tissues of each plant were not found in the male and/or female flower buds (Kumar 1982).

Based on these observations, it may be conjectured that the development of reproductive structures requires some specific proteins varying from species to species. The proteins not found in a particular tissue (vegetative, male or female) may be inhibitory or not necessary for the development of that particular tissue.

5. Role of enzymes and isoenzymes

There are certain enzymes whose activities and/or isozyme patterns differ from vegetative to reproductive phase or from male to female flowers in some species. Retig and Rudich (1972) reported that the activities of peroxidase and IAA oxidase were higher in gynoeious than in monoecious plants of cucumber. Champault (1973) found that the administration of IAA to genetically female plants led to the appearance of male flowers and male-specific isoperoxidases in *Mercurialis annua*. In tissue cultures of *M. annua*, the expression of maleness or femaleness was established by screening for two isoperoxidases which constituted the specific marker for the female strain. Champault *et al* (1981) subsequently called them 'sex-related markers'. Durand *et al* (1974) found certain isoesterases in female flowers of *Mercurialis*, whereas some isoperoxidases were specific to male flowers only. Bazin *et al* (1975) reported two isoesterases to be specific for the female flowers.

Analyses of the peroxidase activity and isoperoxidase patterns have been made in *M. nigra*, *C. papaya*, *C. indica* and *R. communis* (Jaiswal and Kumar 1980b, 1981, 1983; Kumar 1982). In *M. nigra*, *C. papaya* and *C. indica*, the level of peroxidase activity recorded was higher in female flower buds as compared to male flower buds and vegetative tissues. But in *R. communis*, male flower buds had a higher activity than the female flower buds. In *M. nigra*, one isozyme characteristic to vegetative tissues, disappeared in both male and female flower buds and a new band appeared in the male

female flowers have a higher peroxidase activity than male flowers and that 3 isoperoxidases were specific to male flowers only. It is concluded that a higher total peroxidase activity in female flower buds of the dioecious plant indicates its involvement in differentiation of the latter, whereas the larger number of isozymes in male flowers may have a role in male sex expression. However, in *R. communis* just the opposite mechanism seems to be operative.

On the basis of differences in endogenous levels of hormones, discussed above, it may be concluded that control of sex expression operates through the production of specific hormonal substances, the continued synthesis of which is necessary to maintain the phenotypic expression of the genetic sex. Nevertheless, no single hormone has been recognized as solely responsible for regulating a particular sex so far. It is likely that the phenotypic expression of flower sex is controlled by a balance between male-promoting and female-promoting hormones. In several species, femaleness seems to be controlled by the endogenous level of ethylene and maleness by the endogenous level of gibberellins. The plants of either sex seem to be capable of synthesizing both hormones, but the determination of the ultimate sex depends on the balance between the two. If the level of gibberellin is relatively higher than that of ethylene, maleness would be expressed and if ethylene level exceeds that of gibberellins, the shift would be towards femaleness (Mohan Ram and Jaiswal 1974). However, Trewavas and Cleland (1983) have suggested that instances of poor correlation between concentrations of plant hormones found and changes in development may be due to changes in the sensitivity of the concerned tissues to the hormones. Nevertheless, some investigators are of the opinion that the sex expression in plants is controlled by a balance between floral stimulus or flower forming substance and phytohormones (Leonard *et al* 1981; Takahashi *et al* 1982).

Differentiation of organs involves selective gene expression. The right type of gene(s) must be expressed in the particular cells at the right time (Wareing 1982), which results in differences in quality and/or quantity of certain macromolecules of the differentiating cells. During initiation of flower primordia, cells responsible for the development of sex organs acquire biochemical capabilities that are profoundly different from those of the vegetatively growing apical meristems. Compounds such as amino acids, proteins and enzymes/isozymes, which have been found to vary during the reproductive phase, seem to play a prominent role in plant/flower sex differentiation and more information is required to further analyse their role.

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References

- Adams D O and Yang S F 1979 Ethylene biosynthesis: Identification of 1-aminocyclopropane-1-carboxylic acid as an intermediate in the conversion of methionine to ethylene; *Proc. Natl. Acad. Sci. USA* **76** 170-174

- Atsmon D, Lang A and Light E N 1968 Content and recovery of gibberellins in monoecious and gynoeious cucumber plants; *Plant Physiol.* **43** 806–810
- Barber J J and Steward F C 1968 The proteins of tulip and their relation to morphogenesis; *Dev. Biol.* **17** 326–349
- Bazin M, Chabin A and Durand R 1975 Comparaison des taux d' amino-acylation des tRNAs leucine, valine, sérine et tyrosine extraits des fleurs mâles et femelles de *Mercurialis annua* L. en présence des enzymes homologues ou hétérologues; *Plant Sci. Lett.* **4** 207–216
- Bufler G, Mor Y, Reid M S and Yang S F 1980 Changes in 1-aminocyclopropane-1-carboxylic acid content of cut corolla flowers in relation to their senescence; *Planta* **150** 439–442
- Byers R E, Baker L R, Sell H M, Herner R C and Dilley D R 1972 Ethylene: a natural regulator of sex expression of *Cucumis melo* L; *Proc. Natl. Acad. Sci. USA* **69** 717–720
- Chailakhyan M Kh 1968 Flowering hormones of plants; in *Biochemistry and Physiology of Growth Substances* (eds) F Wightman and G Setterfield (Canada: The Runge Press Ltd) pp 1317–1340
- Chailakhyan M Kh and Khryanin V N 1979 Hormonal regulation of sex expression in plants; in *Plant Growth Substances* (ed.) F Skoog (Berlin, Heidelberg, New York: Springer-Verlag) pp 331–344
- Champault A 1973 Effect de quelques régulateurs de la croissance sur des noeuds isolés de *Mercurialis annua* L. ($2n = 16$) cultivés *in vitro*; *Bull. Soc. Bot. Fr.* **120** 87–100
- Champault A, Chung S, Guerin B, Kahlem G, Lhermitte A, Teller G and Durand B 1981 Towards an understanding of the mechanism of cytokinin activity in *Mercurialis annua* L. sex differentiation; in *Metabolism and Molecular Activities of Cytokinins* (eds) J Guern and C Peaud-Lenôel (Heidelberg, New York: Springer-Verlag) 129–139
- Charles- Edwards D A, Cockshull K E, Horridge J S and Thornley J H M 1979 A model of flowering in *Chrysanthemum*; *Ann. Bot.* **44** 557–566
- Dauphin-Guerin B, Teller G and Durand B 1980 Different endogenous cytokinins between male and female *Mercurialis annua* L. *Planta* **148** 124–129
- De-Jong A W and Bruinsma J 1974 Pistil development in *Cleome* flowers III. Effects of growth-regulating substances on flower buds of *Cleome iberidella* Welw. ex. Oliv. grown *in vitro*; *Z. Pflanzenphysiol.* **73** 142–151
- Durand B, Durand R, Kahlem G and Champault A 1974 Interaction moléculaires contrôlant la formation des organes sexuels chez une plante dioïque *Mercurialis annua* L. ($2n = 16$); *Rev. Cyl. Biol. Veg.* **37** 249–256
- Fowden L 1973 Amino acids; in *Phytochemistry* (ed.) L P Miller (New York, Cincinnati, Toronto, London, Melbourne: van Nostrand Reinhold Company) vol. 2, pp 1–29
- Frankel R and Galun E 1977 *Pollination mechanisms, reproduction and plant breeding* (Berlin, Heidelberg, New York: Springer Verlag)
- Friedlander M, Atsmon D and Galun E 1977 Sexual differentiation in cucumber: abscisic acid and gibberellic acid contents of various sex genotypes; *Plant Cell Physiol.* **18** 681–691
- Galun E, Izhar S and Atsmon D 1965 Determination of relative auxin content in hermaphrodite and andromonoecious *Cucumis sativus*; *Plant Physiol.* **40** 321–326
- Ghosh S and Basu P S 1983 Hormonal regulation of sex expression in *Momordica charantia*; *Physiol. Plant.* **57** 301–305
- Gregg K B 1982 Sunlight enhanced ethylene evolution by developing inflorescences of *Catsetum* and *Cynochos* and its relation to female flower production; *Bot. Gaz.* **143** 466–475
- Hayashi F, Boerner D R, Peterson C E and Sell H M 1971 The relative content of gibberellin in seedlings of gynoeious and monoecious cucumber (*Cucumis sativus*); *Phytochemistry* **10** 57–62
- Heslop-Harrison J 1972 Sexuality in angiosperms; in *Plant Physiology—A Treatise* (ed.) F C Steward (New York: Academic Press) vol. 6C, pp 133–289
- Hoffman N E and Yang S F 1980 Changes of 1-amino-cyclopropane-1-carboxylic acid content in ripening fruits in relation to their ethylene production rates; *J. Am. Soc. Hortic. Sci.* **105** 492–495
- Hume B and Lovell P 1982 Female flower production in *Cucurbita pepo* is controlled by ACC (1-aminocyclopropane-1-carboxylic acid) and not by ethylene; *Suppl. Plant Physiol.* **69** 137
- Jaiswal V S and Kumar A 1978 Flower development and amino acid metabolism in some dioecious plants; *Adv. Front. Plant Biol.* Varanasi, pp 6–7
- Jaiswal V S and Kumar A 1980a Role of specific α - amino acids in sex differentiation of *Ricinus communis* L.; *Z. Pflanzenphysiol.* **100** 467–471
- Jaiswal V S and Kumar A 1980b Changes in peroxidase and its multiple forms in relation to sex differentiation in *Coccinia indica*; *Biochem. Physiol. Pflanzen* **175** 580–583

- Jindal K K and Singh R N 1976 Electrophoretic changes in soluble proteins during vegetative and floral development of male and female papaya plants; *Biochem. Physiol. Pflanzen* **170** 301–307
- Kahlem G 1970 Proteines spécifiques et différenciation morphologique chez *Acalypha ornata* Hochst. et *Mercurialis annua* L. ($2n = 16$); *C. R. Acad. Sci. (Paris)*, Series D270, 1314–1317
- Kahlem G 1973 Proteins and development in a dioecious plant: *Mercurialis annua* L.; *Z. Pflanzenphysiol.* **69** 377–380
- Kumar A 1982 *Some aspects of physiology and biochemistry of sex expression in flowering plants*, Ph.D. Thesis, Banaras Hindu University, Varanasi
- Lang A 1952 Physiology of flowering; *Ann. Rev. Plant Physiol.* **3** 265–306
- Leonard M, Kinet J M, Bodson M, Havelange A, Jacquard A and Bernier G 1981 Flowering in *Xanthium strumarium*, Initiation and development of female inflorescence and sex expression; *Plant Physiol.* **67** 1245–1249
- Leshem Y and Ophir D 1977 Differences in endogenous levels of gibberellin activity in male and female partners of two dioecious tree species; *Ann. Bot.* **41** 375–379
- Mifflin B J and Lea P J 1976 The pathway of nitrogen assimilation in plants; *Phytochemistry* **15** 873–885
- Mohan Ram H Y and Jaiswal V S 1974 The possible role of ethylene and gibberellins in flower sex differentiation of *Cannabis sativa*; in *8th International Conf. on Plant Growth Substances* (ed.) Y Sumiki (Tokyo: Hirokawa Publ. Co.) pp 987–996
- Mohan Ram H Y 1980 Hormones and flower sex; *Plant Biochem. J.* (S M Sircar memorial volume) pp 77–88
- Pierard D, Jacquard A and Bernier G 1977 Changes in the protein composition of the shoot apical bud of *Sinapis alba* in transition to flowering; *Physiol. Plant* **41** 254–258
- Retig N and Rudich J 1972 Peroxidase and IAA oxidase activity and isozyme patterns in cucumber plants as affected by sex expression and ethephon; *Physiol. Plant* **27** 156–160
- Rudich J and Halevy A H 1974 Involvement of abscisic acid in the regulation of sex expression in cucumber; *Plant Cell Physiol.* **15** 635–642
- Rudich J, Baker L R, Scott J W and Sell H M 1976 Phenotypic stability and ethylene evolution in androdioecious cucumber; *J. Am. Soc. Hortic. Sci.* **101** 48–51
- Sriram N and Mohan Ram H Y 1984 Sex associated differences in peroxidases and ethylene production and their modification by ethephon treatment in the flowers of *Cannabis sativa* L.; *Curr. Sci.* **53** 735–739
- Takahashi H, Saito T and Suge H 1982 Intergeneric translocation of floral stimulus across a graft in monoecious cucurbitaceae with special reference to the sex expression of flowers; *Plant Cell Physiol.* **23** 1–9
- Thornley J H M 1972 A model of a biochemical switch, and its application to flower initiation; *Ann. Bot.* **36** 861–871
- Trewavas A J and Cleland R E 1983 Is plant development regulated by changes in the concentration of growth substances? *Trends Biochem. Sci.* **8** 354–356
- Umbarger H E 1978 Amino acid biosynthesis and its regulation; *Ann. Rev. Biochem.* **47** 533–606
- Vince-Prue D 1975 *Photoperiodism in plants*; (Maidenhead, Berkshire, England: McGraw Hill Book Co. Ltd.) pp 312–330
- Wareing P F 1982 Determination and related aspects of plant development; in *The Molecular Biology of Plant Development* (eds) H Smith and D Grierson (Oxford, London, Edinburgh, Boston, Melbourne: Blackwell Scientific Publications) pp 517–541

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